UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 10-MAR-2004

Subject: Acequinocyl. Registration for Use on Pome Fruits, Citrus, Almonds, Pistachios, and Strawberries. Summary of Analytical Chemistry and Residue Data.

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Note: This document was originally prepared under contract by Dynamac Corporation (1910 Sedwick Road, Building 100, Suite B, Durham, NC 27713; submitted 09-APR-2003). The document has been reviewed by the HED and revised to reflect current OPP policies.

Executive Summary

Arvesta Corporation has submitted a petition proposing the use of acequinocyl [2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione] (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal flowable concentrate (FlC)), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. Acequinocyl has been given a reduced-risk status. There are currently no food/feed uses or tolerances for acequinocyl. Acequinocyl is registered for use on greenhouse, shadehouse, ornamental, floral, foliage and nursery crops.

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Data from the plant and ruminant metabolism studies were presented to the HED Metabolism Assessment Review Committee (MARC) for determination of the residues of concern (Memo, S. Levy, et.al., 07-JAN-2004; TXR #: 0052294; see Attachment 2 for chemical structures and identification of compounds). The nature of acequinocyl residues in fruit crops is understood based on adequate apple, orange, and eggplant metabolism studies. In each of these studies, the major ^{14}C-residue in/on various matrices was identified as parent compound. The available data indicate that the metabolism of acequinocyl in these crops involves the loss of the acetyloxy moiety to form acequinocyl-OH (also referred to as Metabolite R1), opening of the quinone ring to form AKM-18, and subsequent degradation of the quinone ring to yield polar metabolites that degrade to phthalic acid. The HED MARC determined that parent and acequinocyl-OH are the residues of concern for risk assessment and tolerance expression. The plant metabolism studies on apples, oranges, and eggplants indicated that parent is the major residue in all matrices, with no metabolites >10% of the total radioactive residue (TRR). Acequinocyl-OH was found as a minor metabolite in most matrices except in eggplant fruit where it accounts for 10.3% of the TRR. However, acequinocyl-OH was analyzed in field trials and was above the limit of quantitation (LOQ) in some matrices. For example, in almond hulls acequinocyl-OH was detected at 0.06 ppm (LOQ = 0.01 ppm). Based on the structural similarity to the parent, MARC considers acequinocyl-OH to share similar toxicity as the parent (*i.e.*, it still possesses the naphthoquinone moiety believed to be responsible for the blood coagulation effects). Since the analytical method detects both parent and acequinocyl-OH, the MARC concluded that parent and acequinocyl-OH are the residues of concern for risk assessment and tolerance expression.

The qualitative nature of acequinocyl residues in ruminants is adequately understood based on the adequate goat metabolism study. The metabolism of acequinocyl in goats appears to involve loss of the acetyloxy moiety to form acequinocyl-OH and partial cleavage of the dodecyl side chain to form AKM-15. Opening and degradation of the quinone ring was also evidenced by the presence of AKM-18 and phthalic acid. As none of the proposed uses include crops having poultry feed items, poultry metabolism and feeding studies are not required for the current petition. The goat metabolism study indicated that parent or acequinocyl-OH is the major residue. Minor metabolites include AKM-18 (1.8% TRR in liver and 6.2% TRR in kidney) and AKM-15 (9% of the TRR in liver and 9.1% in kidney). The MARC determined that since both acequinocyl-OH and AKM-15 share a similar structure (the naphthoquinone) as the parent, then they are therefore considered to share similar toxicity. AKM-15 is also present in kidney and liver at levels comparable to parent and/or acequinocyl-OH. Since the analytical method detects parent and acequinocyl-OH, the MARC concluded that for tolerance expression, parent and acequinocyl-OH are the residues of concern. For risk assessment, parent, acequinocyl-OH and AKM-15 (in liver and kidney only) need to be included. AKM-18 is of less toxicological concern than the parent and acequinocyl-OH, and therefore, AKM-18 can be excluded.

Arvesta is presently proposing permanent tolerances for residues of acequinocyl in/on almond nutmeats (0.01 ppm) and hulls (1.5 ppm), pome fruits (0.4 ppm), wet apple pomace (1.0 ppm), citrus fruits (0.3 ppm), orange oil (30 ppm), pistachio nutmeats (0.01 ppm), strawberries (0.4 ppm), milk (0.01 ppm), and fat (0.02 ppm), kidney (0.01 ppm), liver (0.02 ppm), and muscle (0.01 ppm) of cows.

The submitted apple and pear field trial data are adequate and reflect the proposed use pattern for pome fruits. The residue data for apples and pears support the proposed 0.4 ppm tolerance for residues in/on the pome fruits. The available almond, strawberry, grapefruit, lemon and orange field trials also reflect the respective proposed use patterns. The almond data are translated to support a tolerance on pistachio. The strawberry data support a tolerance of 0.40 ppm. The available grapefruit, lemon, and orange field trial data support a tolerance of 0.20 ppm in/on citrus fruits and the almond field trail data support tolerances of 0.02 ppm for almond and pistachio and 2.0 for almond, hulls.

An adequate apple processing study was submitted to support the proposed use on pome fruits. As the processing factor for apple juice (0.03x) was ≤1x, a tolerance is not required for apple juice. However, residue concentrations were observed in wet apple pomace (3.5x). Based on the observed 3.5x processing factor for wet apple pomace and the highest average field trial (HAFT) residues of 0.213 ppm from the apple field trials, the maximum expected acequinocyl residues in wet apple pomace would be 0.75 ppm. These data support a tolerance of 1.0 ppm for residues in wet apple pomace.

An adequate orange processing study was submitted to support the proposed use on citrus fruits. The study indicates that combined residues of acequinocyl did not concentrate appreciably in orange juice (0.04x) or dried pulp (1.09x). Therefore, separate tolerances would not be required for these commodities. However, residue concentrations were observed in citrus oil (165x). Based on the 165x processing factor for oil and HAFT residues of 0.174 ppm from the orange field trials, the maximum expected acequinocyl residues in citrus oil would be 28.7 ppm. These data support a tolerance of 30 ppm for residues in citrus oil.

The available apple storage stability data indicate that acequinocyl and acequinocyl-OH are stable in frozen (-20 C) apple commodities for at least 5 months; these data support the pome fruit and strawberry field trials and the apple processing study. The available orange storage stability data indicate that acequinocyl and acequinocyl-OH are stable in frozen (-20 C) citrus fruit for at least 5 months (3 months for citrus processed commodities); these data support the citrus field trials and orange processing study. The available almond storage stability data indicate that acequinocyl and acequinocyl-OH are stable in frozen (-20 C) almonds for up to 3.5 months; these data support the almond field trial study. **To support the cattle feeding study, data are required depicting the stability of acequinocyl and acequinocyl-OH residues in milk and tissue samples frozen for up to 250 days.**

There are no methods currently listed in the Pesticide Analytical Manual (PAM) Vol. II for determining acequinocyl residues in or on plant or livestock commodities. Arvesta has submitted method validation data for the following two plant methods and a livestock method: a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse Laboratories Method #Meth-133, revision #3) for determining residues of acequinocyl and acequinocyl-OH in/on fruit commodities; an HPLC/MS/MS method (Morse Laboratories Method #Meth-135) for determining residues of acequinocyl and acequinocyl-OH in/on nutmeats and almonds hulls; and an HPLC/MS/MS method (Morse Laboratories Method #Meth-139, Revision

#2) for determining residues of acequinocyl and acequinocyl-OH in milk, meat, meat-by-products, and fat.

Based on the available method validation data (discussed below), these methods are adequate for collecting residue data in/on pome and citrus fruit commodities, tree nuts, and milk and livestock commodities. The validated LOQ for both acequinocyl and acequinocyl-OH is 0.01 ppm in/on each plant and livestock commodity, with the exception of citrus oil. The LOQ for each analyte in citrus oil is 0.5 ppm.

Methods #Meth-135 and #Meth-133, Revision #3 have each undergone successful independent laboratory validation (ILV) trials (MRIDs 45651602 and 45782301, respectively). An ILV was not submitted by the petitioner for Method #Meth-139, Revision #2; however, because of the similarity of all three methods, the aforementioned ILVs should be sufficient to cover this method. Methods #Meth-135 and #Meth-133, Revision #3, and #Meth-139, Revision #2 were forwarded to the Analytical Chemistry Branch (ACB) for a petition method validation (PMV) (Memo, S. Levy, 19-NOV–2003; D296183).

None of these methods were radiovalidated using samples from the submitted plant or goat metabolism studies. As the analytical method for fruits (#Meth-133) used the same solvent extraction as the apple and orange metabolism studies, radiolabeled method validation data are not required for this method. After personal communication with ACB (18-FEB-2004; C. Stafford to S. Levy), ACB determined the above HPLC/MS/MS methods only have one transition (one ion to one ion); therefore, these methods cannot serve as confirmatory methods. **Additional confirmatory methods for plants and livestock and specificity testing of the analytical enforcement methods for plants and livestock are required.**

The submitted cattle feeding study is adequate, contingent upon submission of an adequate livestock storage stability study. In the feeding study, cows were dosed orally with acequinocyl at levels equivalent to 5, 15, or 50 ppm in the diet. These levels are equivalent to 6.9x, 21x, and 69x the maximum theoretical dietary burden (MTDB) for dairy cattle (0.72 ppm) and 4.1x, 12x, and 41x the MTDB for beef cattle (1.22 ppm).

Although tentative, data from the feeding study indicate that tolerances will not be required for milk, kidney, and meat of ruminants. Combined acequinocyl residues in milk and muscle were below the combined LOQ (<0.02 ppm) in all samples from all three dose groups (up to 69x the MTDB for dairy cattle and up to 41x the MTDB for beef cattle) and combined residues were <LOQ in kidneys from the 4.1 and 12x dose groups. However, quantifiable residues of at least acequinocyl-OH were found in liver and fat samples from the 4.1x and 12x dose groups; therefore tolerances are required for these commodities. Considering the maximum combined residues observed in liver (<0.032 ppm) and fat (<0.013 ppm) from the 5 ppm dose group (4.1x MTDB), the maximum expected residues in liver and fat resulting from 1x the MTDB would be below the combined LOQ. Accordingly, the tolerances for residues in these commodities (liver and fat) of cattle, goat, horse, and sheep should be set at the LOQ (0.02 ppm) for the analytical method.

In addition, tolerances are not required for any hog commodities as none of the proposed uses include crops having hog feed items. Furthermore, a confined or field rotational crop study were not submitted with these petitions. As strawberries are rotated crops, a confined rotational crop study should be submitted. Until an acceptable study is submitted, rotation should be prohibited to any crop other than strawberries. **A revised Section B should be submitted.**

Residue Chemistry Deficiencies

860.1200 Directions for Use

1. As strawberries are rotated crops, a confined rotational crop study should be submitted. Until an acceptable study is submitted, rotation should be prohibited to any crop other than strawberries. A revised Section B should be submitted.

OPPTS 860.1340 Residue Analytical Methods

2a. Methods #Meth-135, #Meth-133, Revision #3, and #Meth-139, Revision #2 will undergo a PMV at ACB/BEAD (Memo, S. Levy, 19-NOV–2003; D296183). Successful completion of the PMVs are necessary before the proposed methods can be employed for enforcement purposes.

2b. After personal communication with ACB (18-FEB-2004; C. Stafford to S. Levy), ACB determined the HPLC/MS/MS methods only have one transition (one ion to one ion); therefore, these methods cannot serve as confirmatory methods. Additional confirmatory methods for plants and livestock and specificity testing of the analytical enforcement methods for plants and livestock are required.

860.1380 Storage Stability

3. Under the conditions and parameters used in the livestock storage stability study, the data are classified as scientifically unacceptable. A new livestock storage stability study should be submitted. As Day-0 samples were not analyzed, a determination of residue levels present at the time samples were placed in frozen storage could not be made. An insufficient number of time points were analyzed in order to establish that residues of acequinocyl or acequinocyl-OH were stable throughout duration of the study or to show how much of the residue was lost at various time points.

860.1480 Meat, Milk, Poultry, and Eggs

4. In the ruminant feeding study, total frozen storage intervals were 119-242 days for whole milk and 168-252 days for all tissues. The ruminant feeding study is considered acceptable, pending submission of an acceptable livestock storage stability study (data depicting the stability of acequinocyl and acequinocyl-OH residues in milk and tissue samples frozen for up to 250 days are needed).

860.1650 Submittal of Analytical Reference Standards

5. The analytical reference standard for acequinocyl-OH has not been submitted to the EPA National Pesticide Standards Repository (electronic communication, S. Levy and C. Stafford to T. Cole, 08-JAN-2003). This is a deficiency.

860.1850/1900 Confined and Field Accumulation in Rotational Crops

6. A confined or field accumulation study were not submitted with these petitions. As strawberries are rotated crops, a confined rotational crop study should be submitted.

860.1550 Proposed Tolerances

7a. The petitioner should submit a revised Section F. The HED MARC determined that acequinocyl and acequinocyl-OH are the residues of concern for tolerance expression. [Arvesta proposed the establishment of permanent tolerances for residues of acequinocyl in/on plant and livestock commodities on the Section F (although the draft filing notices for strawberries and pome fruits state acequinocyl + acequinocyl-OH, expressed as parent equivalents (electronic mail, M. Mautz to S. Levy, 12-DEC-2003)).]

7b. The available apple and pear field trial data and the apple processing study support the proposed tolerances for the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) in/on pome fruits at 0.40 ppm and in wet apple pomace at 1.0 ppm. The grapefruit, lemon, and orange field trial data support a tolerance for the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) of 0.20 ppm for citrus fruits, and the almond field trail data support tolerances for the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) of 0.02 ppm for almond (method LOQ for combined residues) and 2.0 for almond, hulls. In addition, the orange processing data support a tolerance for the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) of 30 ppm tolerance for citrus oil. The almond data are translated to support a tolerance for the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) of 0.02 ppm for pistachio.

7c. Although additional storage stability data are required to upgrade the cattle feeding study to adequate, data from the feeding study suggest that tolerances will not be necessary for residues in milk, meat, and kidneys of ruminants, as there is no reasonable expectation of finding quantifiable residues in these commodities [40 CFR 180.6(a)(3)] even at level up to 10x the MTDB. However, tolerances are likely to be required for combined acequinocyl residues in fat and liver of cattle, goat, horse, and sheep. If the requested storage stability data indicate that residues are stable in frozen livestock commodities, then tolerances for the combined acequinocyl residues in liver and fat should be set at the LOQ (0.02 ppm) for the analytical method.

RECOMMENDATIONS

Provided that revised Sections B and F are submitted, an analytical reference standard for acequinocyl-OH is submitted to the EPA National Pesticide Standards Repository, and successful PMV's for analytical methods #Meth-135, #Meth-133, Revision #3, and #Meth-139, Revision #2 are conducted, HED concludes there are no residue chemistry data requirements that would preclude the establishment of the following permanent tolerances for residues of acequinocyl and acequinocyl-OH (R1):

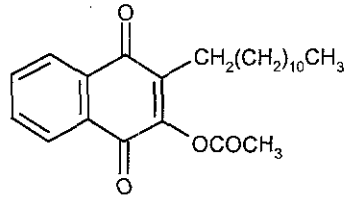
Almond	0.02
Almond, hulls	2.0
Apple, wet pomace	1.0
Fruit, Citrus, Group 10	0.20
Fat of cattle, goat, horse, and sheep	0.02
Liver of cattle, goat, horse, and sheep	0.02
Citrus, oil	30
Pistachio	0.02
Fruit, Pome, Group 11	0.40
Strawberry	0.40

Registration of Kanemite™ may be made permanent with submission of the following:

- Confined Rotational Crop Study.
- A new livestock storage stability study.
- Confirmatory Methods for Plants and Livestock.
- Specificity Testing of Analytical Enforcement Methods for Plants and Livestock.
- Data depicting the stability of acequinocyl and acequinocyl-OH residues in milk and tissue samples frozen for up to 250 days.

A human-health risk assessment will be prepared as a separate document.

Acequinocyl



Background

Acequinocyl [2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione] is a quinoline type miticide proposed for control of the Two-spotted spider mite (*Tetranychus urticae*) and European red mite (*Panonychus ulmi*) on almonds, pistachios and pome fruit; Citrus red mite (*Panonychus citric*) and Texas citrus mite (*Eutetranychus banski*) on citrus; and Two-spotted spider mite (*Tetranychus urticae*) and Strawberry spider mite (*Tetranychus turkestani*) on strawberries. Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ SC, 1.25 lb/gal FlC) in the United States on pome fruits, citrus fruits, almonds, pistachios, and strawberries. There are currently no food/feed uses or tolerances for acequinocyl. In conjunction with this use, Arvesta is proposing the establishment of permanent tolerances for residues of acequinocyl in/on the following raw plant and livestock commodities:

Almond, nutmeat	0.01 ppm
Almond, hulls	1.5 ppm
Apple, wet pomace	1.0 ppm
Citrus Crop Group	0.3 ppm
Cow, fat	0.02 ppm
Cow, kidney	0.01 ppm
Cow, liver	0.02 ppm
Cow, muscle	0.01 ppm
Milk	0.01 ppm
Orange, oil	30 ppm
Pistachio, nutmeat	0.01 ppm
Pome Fruit Crop Group	0.4 ppm
Strawberries	0.4 ppm

860.1200 Directions for Use

Table 1. Summary of Directions for Use of Acequinocyl.

Trade Name	Applic. Timing, Type, and Equip.	Applic. Rate (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A)	PHI (days)	Use Directions and Limitations
Citrus, Almonds and Pistachios						
Kanemite™ 15 SC 1.25 lb/gal SC [66330-xxx]	Broadcast foliar application Ground equipment	0.3	2	0.6	7	The proposed label specifies a minimum retreatment interval (RTI) of 21 days and a maximum seasonal use rate of 0.6 lb ai/A. A minimum application volume of 100 gallons/A is specified. Applications through irrigation systems or using aerial equipment are prohibited.
Pome Fruits						
Kanemite™ 15 SC 1.25 lb/gal SC [66330-xxx]	Broadcast foliar application Ground equipment	0.3	2	0.6	14	The proposed label specifies a RTI of 21 days and a maximum seasonal use rate of 0.6 lb ai/A. A minimum application volume of 100 gallons/A is specified. Applications through irrigation systems or using aerial equipment are prohibited.
Strawberries						
Kanemite™ 15 SC 1.25 lb/gal SC [66330-xxx]	Broadcast foliar application Ground equipment	0.3	2	0.6	1	The proposed label specifies a RTI of 21 days and a maximum seasonal use rate of 0.6 lb ai/A. A minimum application volume of 100 gallons/A is specified. Applications through irrigation systems or using aerial equipment are prohibited.

[a] The proposed label specifies a restricted-entry interval (REI) of 12 hours.

The proposed use directions adequately reflect the use pattern from the pome fruit, citrus fruit, almond, and strawberry field trials. The almond field trial data will be used to support the use on pistachios. A confined or field rotational crop study were not submitted with these petitions.

As strawberries are rotated crops, a confined rotational crop study should be submitted. Until an acceptable study is submitted, rotation should be prohibited to any crop other than strawberries. **A revised Section B should be submitted.**

860.1300 Nature of the Residue - Plants

The qualitative nature of acequinocyl residues in fruit crops is understood based on the apple, orange, and eggplant metabolism studies submitted with the current petition. The available data indicate that the metabolism of acequinocyl in these crops involves the loss of the acetyloxy moiety to form acequinocyl-OH, opening of the quinone ring to form AKM-18, and subsequent degradation of the quinone ring to yield polar metabolites that degrade to phthalic acid.

Data from the plant metabolism studies were presented to the HED MARC for determination of the residues of concern in plant commodities (Memo, S. Levy, et.al., 07-JAN-2004; TXR #: 0052294). Plant metabolism studies on apples, oranges, and eggplants indicated that parent is the major residue in all matrices, with no metabolites >10% of the TRR. Acequinocyl-OH was found as a minor metabolite in most matrices except in eggplant fruit where it accounts for 10.3% of the TRR. However, acequinocyl-OH was analyzed in field trials and was above the LOQ in some matrices. For example, in almond hulls acequinocyl-OH was detected at 0.06 ppm (LOQ=0.01 ppm). Based on the structural similarity to the parent, MARC considers acequinocyl-OH to share similar toxicity as the parent (*i.e.*, it still possesses the naphthoquinone moiety believed to be responsible for the blood coagulation effects). Since the analytical method detects both parent and acequinocyl-OH, MARC concluded that parent and acequinocyl-OH are the residues of concern for risk assessment and tolerance expression.

45651701.der

In an apple metabolism study, [U-^{14}C-phenyl]- or [1-^{14}C-dodecyl]acequinocyl (≥95% radiochemical purity) was formulated as a FlC and applied once as a broadcast foliar application during fruit development to apple trees grown outdoors in containers. [^{14}C]Acequinocyl was applied at rates equivalent to 0.76-0.776 kg ai/ha (0.637-0.691 lb ai/A). Fruit samples were collected at 0, 14, 21, and 30 days after treatment (DAT) and leaf samples were collected at 0 and 30 DAT.

The levels of TRRs in/on apple fruits and leaves were generally similar for the two ^{14}C-labels. On the day of application, average TRRs were 1.302 and 1.386 ppm in/on fruits and 53.92 and 54.13 ppm in/on leaves from both ^{14}C-labels. Decline in TRR values were variable. TRR in/on [^{14}C-PH]-treated fruits initially declined to 0.384 ppm at 14 DAT, but was 0.584-0.592 ppm by 21 and 30 DAT. For the [^{14}C-DOD]-treated fruits, TRR declined to 0.668-0.698 ppm by 14-30 DAT. For leaves, TRR declined to 23.72 ppm in/on [^{14}C-PH]-treated leaves and to 4.6 ppm in/on [^{14}C-DOD]-treated leaves by 30 DAT. Translocation of ^{14}C-residues from leaves to fruits was minimal. When fruits were covered prior to foliar application of either ^{14}C-label, TRR in/on fruits were 0.014-0.016 ppm at 30 DAT.

For both ^{14}C-labels, the majority of the TRR consisted of surface residues. Surface ^{14}C-residues in/on fruit and leaves accounted for the 98.0-98.7% of the TRR at 0 DAT and declined to 39.9-63.2% of the TRR at 30 DAT. The percentage of the fruit TRR associated with the fruit peel and flesh increased over time. By 30 DAT, radioactivity in the peel accounted for 28.6-44.1% of the TRR and radioactivity in the flesh accounted for 8.2-10.5% of the TRR.

Surface washes and solvent extractions released 68.4-99.7% of the TRR from fruits and leaves, and base extractions with 1M and 5M NaOH at 55 C released an additional 13.8-29.5% of the TRR. The overall recovery of radioactivity from fruit and leaf samples was 98.7-100.1%. HPLC and thin-layer chromatography (TLC) analyses identified 48.5-94.0% of the TRR in/on fruits and 35.2-93.5% of the TRR in/on leaves, with ^{14}C-residues being identified by co-chromatography with reference standards. Sufficient information was available to assess the stability of ^{14}C-residues; no additional sample storage information or stability data are required.

The metabolite profile was similar between the two ^{14}C-labels and between fruits and leaves. For fruits, acequinocyl accounted for 88.7-92.0% of the TRR at 0 DAT and 28.4-57.0% of the TRR at 14-30 DAT. Minor amounts of acequinocyl-OH (1.4-4.4% TRR) and AKM-18 (0.1-2.1% TRR) were also identified in fruits at each interval. Phthalic acid was also identified in fruits (~8.5-17.1% TRR) from the 14-30 DAT intervals, although the quantities reported are approximate as TLC separation was not complete. The metabolite 2-carboxy-α-oxo-benzene acetic acid (CBAA) was also tentatively identified in [^{14}C-PH]-labeled fruits as a minor component of the polar residues. The remaining solubilized ^{14}C-residues from fruits were comprised primarily of unknown polar metabolites, each accounting for <5% of the TRR.

For leaves, acequinocyl accounted for 91.2-93.5% of the TRR at 0 DAT and 20.0-26.0% of the TRR by 30 DAT. At both intervals, acequinocyl-OH accounted for 0.9-1.5% of the TRR and AKM-18 accounted for 0.2-4.8% of the TRR. Residues of phthalic acid were also identified as a component of the polar ^{14}C-residues at 30 DAT (10.5-12.4% TRR). Unidentified, extractable ^{14}C-residues accounted for a total of 35.2-44.5% of the TRR by 30 DAT and were comprised primarily of polar metabolites, each accounting for <8% of the TRR.

Based on the identified metabolites, the metabolism of acequinocyl in apples appears to involve the loss of the acetyloxy moiety to form acequinocyl-OH, opening of the quinone ring to form AKM-18, and subsequent degradation of the quinone ring to yield phthalic acid.

45651702.der

In an orange metabolism study, [U-^{14}C-phenyl]-acequinocyl (≥97% radiochemical purity) was formulated as a FlC and applied once as a broadcast foliar application during fruit development to an orange tree growing outdoors. The [^{14}C]acequinocyl was applied at a rate equivalent to 1.05 kg ai/ha (0.94 lb ai/A). Fruit and leaf samples were collected at 0, 14, 21, and 30 DAT.

Average TRR in/on treated fruits declined from 0.633 ppm at 0 DAT to 0.228 ppm by 30 DAT, and average TRR in/on leaves declined from 53.7 ppm at 0 DAT to 25.9 ppm by 30 DAT. Translocation of ^{14}C-residues from leaves to fruits was minimal as indicated by the TRR levels in fruits (0.043 ppm) harvested at 30 DAT from the treated area of tree, but which were covered with plastic bags during the application.

The majority of the TRR in/on fruits and leaves consisted of surface residues, accounting for 97.8% of the TRR immediately following application and declining to 46.9% of the TRR by 30 DAT. ^{14}C-Residue levels in the fruit peel increased over time from 2.2% of the TRR at 0 DAT to

50.5% of the TRR by 30 DAT. ^{14}C-Residues in the flesh were minimal (≤2.95% of the TRR) at all intervals. For leaves, surface ^{14}C-residues accounted for 99.6% of the TRR at 0 DAT and declined to 55.3% of the TRR by 30 DAT.

Surface washes and solvent extractions with acetonitrile (ACN) released 71.5-99.6% of the TRR from fruits and 64.5-99.8% of the TRR from leaves at all sampling intervals. The overall recovery of radioactivity from the fruit and leaf samples was 98.8-103.7%. HPLC and TLC analyses identified 44.8-97.5% of the TRR in fruit and 37.7-99.1% of the TRR in leaves, with ^{14}C-residues being identified by co-chromatography with reference standards. Sufficient information was available to assess the stability of ^{14}C-residues; no additional sample storage information or stability data are required.

The metabolite profile for fruit and leaves was similar, with acequinocyl being the major ^{14}C-residue in both matrices at each sampling interval. At 0 DAT, acequinocyl accounted for 95.1 and 97.9% of the TRR in/on fruits and leaves, respectively. At the later sampling intervals (14, 21, and 30 DAT), acequinocyl accounted for 35.8-41.4% of the TRR in/on fruits and 27.7-36.4% of the TRR in/on leaves. Metabolites acequinocyl-OH and AKM-18 were identified as minor components, with each accounting for ≤2.1% of the TRR in fruits and ≤6.8% of the TRR in leaves. A substantial portion of the TRR in both fruits and leaves was characterized as being comprised of polar components. One of the polar compounds was identified as phthalic acid, accounting for 7.0-8.2% of the TRR in fruits and 4.9-10.5% of the TRR in leaves.

Polar Unknown 1A accounted for 11.3-15.8% of the TRR in fruits and 3.1-7.4% of the TRR in leaves, and polar Unknown 1B accounted for 5.4-7.3% of the TRR in fruits and 7.0-14.5% of the TRR in leaves. Unknown 1A was reported to be CBAA. In summary, individual components in the polar fruit fraction each accounted for <0.05 ppm and were characterized as being acidic. Furthermore, one of the three major components in the polar fraction was identified as CBAA. Six components were detected in the polar fraction of leaves and were characterized as being acidic. Two of the more minor components in the polar fraction were identified as phthalic acid and CBAA.

Based on the identified metabolites, the metabolism of acequinocyl in oranges appears to involve the loss of the acetyloxy moiety to form acequinocyl-OH, opening of the quinone ring to form AKM-18, and subsequent degradation of the quinone ring to yield phthalic acid.

45651703.der

In an eggplant metabolism study, [U-^{14}C-phenyl]- or [1-^{14}C-dodecyl]acequinocyl (≥95% radiochemical purity) was formulated as a FlC and applied once as a broadcast foliar or soil application during fruit development to eggplants grown in growth chambers. The [^{14}C]acequinocyl was applied at rates equivalent to 0.55-0.62 kg ai/ha (0.49-0.55 lb ai/A). Fruit and leaf samples were collected at 0, 7, and 14 DAT.

The levels of TRR in/on eggplant fruits and leaves were quite variable, but were generally similar for the two ^{14}C-labels. For fruits, average TRR were 0.116-0.374 ppm at 0 DAT, 0.087-0.156 ppm at 7 DAT, and 0.061-0.140 ppm at 14 DAT. For leaves, average TRR were 13.98-26.60 ppm at 0 DAT, 13.04-22.60 ppm at 7 DAT, and 5.00-7.21 ppm at 14 DAT. Translocation of ^{14}C-residues from leaves to fruits and uptake from the soil was minimal. When fruits were covered prior to foliar application of either ^{14}C-label, TRR in/on fruits were 0.005-0.031 ppm at 14 DAT. When either ^{14}C-label was applied to soil, TRR were 0.005-0.012 ppm in/on fruit and 0.018-0.031 ppm in/on leaves at 14 DAT.

Following a foliar application, the majority of the TRR consisted of surface residues. Surface ^{14}C-residues in/on fruit and leaves accounted for the 95.1-98.2% of the TRR at 0 DAT and 60.7-79.8% of the TRR at 14 DAT. The percentage of the fruit TRR associated with the fruit peel and flesh increased over time. By 14 DAT, radioactivity in the peel accounted for 18.0-28.6% of the TRR and radioactivity in the flesh accounted for 6.6-11.4% of the TRR.

Surface washes and solvent extractions released 83.6-99.5% of the TRR from fruits and 73.2-99.7% of the TRR from leaves at all sampling intervals. Enzymatic digestion and mild base extraction released an additional 4.8-19.7% of the TRR. The overall recovery of radioactivity from the fruit and leaf samples was 97.1-104.9%. HPLC and TLC analyses identified 53.3-89.8% of the TRR in fruit and 47.5-94.5% of the TRR in leaves, with ^{14}C-residues being identified by co-chromatography with reference standards. Sufficient information was available to assess the stability of ^{14}C-residues; no additional sample storage information or stability data are required.

The metabolite profile in/on fruits and leaves was similar for both the [^{14}C-phenyl]- and [^{14}C-dodecyl]-labels. Acequinocyl was the major ^{14}C-residue in/on fruits and leaves at all sampling intervals. In fruit, acequinocyl accounted for 81.6-83.7% of the TRR at 0 DAT and 46.2-58.4% of the TRR at 7 and 14 DAT; and in leaves, acequinocyl accounted for 89.0-92.1% of the TRR at 0 DAT, 51.8-74.9% of the TRR at 7 DAT, and 38.4-58.1% of the TRR by 14 DAT. Acequinocyl-OH was a minor component of the residue in leaves and fruits at each interval, accounting for 4.3-10.3% of the TRR in fruits and 1.0-4.1% of the TRR in leaves. Minor amounts of AKM-18 (0.3-6.7% TRR) were also identified in fruits and leaves at each interval. The remaining solubilized ^{14}C-residues in leaves and fruits were comprised primarily of minor polar metabolites, each accounting for <10% of the TRR. Although quantitative data were limited, one of the unknown fractions (1C) was identified as phthalic acid, and two other polar unknowns were characterized as minor acidic metabolites that degraded to phthalic acid following base hydrolysis.

Based on the identified metabolites, the metabolism of acequinocyl in eggplants appears to involve the loss of the acetyloxy moiety to form acequinocyl-OH, opening of the quinone ring to form AKM-18, and subsequent degradation of the quinone ring to yield phthalic acid.

860.1300 Nature of the Residue - Livestock

The qualitative nature of acequinocyl residues in ruminants is understood based on the submitted goat metabolism study submitted with the current petition. The metabolism of acequinocyl in goats appears to involve loss of the acetyloxy moiety to form acequinocyl-OH and partial cleavage of the dodecyl side chain to form AKM-15. Opening and degradation of the quinone ring was also evidenced by the presence of AKM-18 and phthalic acid.

Data from the ruminant metabolism study was presented to the HED MARC for determination of the residues of concern in ruminant commodities (Memo, S. Levy, et.al., 07-JAN-2004; TXR #: 0052294). The goat metabolism study indicated that parent or acequinocyl-OH is the major residue. Minor metabolites include AKM-18 (1.8% TRR in liver and 6.2% TRR in kidney) and AKM-15 (9% of the TRR in liver and 9.1% in kidney). Both acequinocyl-OH and AKM-15 share a similar structure (the naphthoquinone) as the parent and therefore the MARC considers them to share similar toxicity. AKM-15 is also present in kidney and liver at levels comparable to parent and/or acequinocyl-OH. Since the analytical method detects parent and acequinocyl-OH, the MARC concluded that for tolerance expression, parent and acequinocyl-OH are the residues of concern. For risk assessment, parent, acequinocyl-OH and AKM-15 (in liver and kidney only) needed to be included. AKM-18 is of less toxicological concern than the parent, and acequinocyl-OH, and therefore, AKM-18 can be excluded.

As none of the proposed uses include crops having poultry feed items, a poultry metabolism study is not required for the current petition.

45651704.der

In a ruminant metabolism study, a single dairy goat was dosed orally, via capsules, once a day for 5 consecutive days with [U-^{14}C-phenyl]-acequinocyl (>96.7% radiochemical purity) at 17.66 mg/day, equivalent to 0.28 mg/body weight/day. Based on actual feed consumption, this dose level was equivalent to 11.3 ppm of [^{14}C]acequinocyl in the diet (16x the MTDB). Although the goat used in the study was dosed at the minimum level recommended by the Agency, the resulting levels of radioactivity in milk and tissues were too low to provide adequate identification of the ^{14}C-residues.

A total of 85.7% of the administered dose was recovered, with the majority of the dose being recovered in the feces (64.2% dose) and G.I. tract (10.8% dose). Another 9.9% of the administered dose was recovered in the urine. Radioactivity remaining in edible tissues at sacrifice accounted for 0.7% of the dose and <0.1% of the dose was excreted in the milk. Maximum levels of radioactivity in milk were 0.0027 ppm (Day 5), and TRR in tissues were relatively low at 0.14 ppm in liver, 0.10 ppm in kidneys, 0.017-0.018 ppm in fat, and 0.006-0.008 ppm in muscle.

Solvent extraction released 55.7-77.5% of the TRR from liver, kidney and fat, and protease digestion released an additional 12.9-23.9% of the TRR from kidneys and liver. Radioactivity remaining in the residual solids from each tissue accounted for <0.03 ppm, and the overall recovery of radioactivity from tissues was ~100%.

Characterization of ^{14}C-residues in liver, kidneys and fat was limited, and quantitation of ^{14}C-residues was problematic due to poor separation of components. In liver, parent (1.5% TRR) was detected along with metabolites acequinocyl-OH (8.4% TRR), AKM-15 (9.0% TRR), and AKM-18 (1.8% TRR). Parent (10.3% TRR) was also detected in kidneys along with metabolites acequinocyl-OH (<1% TRR), AKM-15 (9.1% TRR), and AKM-18 (6.2% TRR). In fat, parent (21.5% TRR) was detected along with acequinocyl-OH (22.5% TRR). Supporting analyses of feces and urine detected parent (14.4%) and acequinocyl-OH (9.3%) and AKM-18 (27.1%) in feces and phthalic acid (33-42%) in urine. TRR in milk were <0.003 ppm and in muscle were less than 0.01 ppm. As the TRR in these samples were <0.01 ppm, no further investigations were carried out.

The metabolism of acequinocyl in goats appears to involve loss of the acetyloxy moiety to form acequinocyl-OH and partial cleavage of the dodecyl side chain to form AKM-15. Opening and degradation of the quinone ring was also evidenced by the presence of AKM-18 and phthalic acid.

860.1340 Residue Analytical Methods

There are no methods currently listed in the PAM Vol. II for determining acequinocyl residues in or on plant or livestock commodities. In the current petition, Arvesta has submitted method validation data for the following two plant methods and an livestock method: an HPLC/MS/MS method (Morse Laboratories Method #Meth-133, revision #3) for determining residues of acequinocyl and acequinocyl-OH in/on fruit commodities; an HPLC/MS/MS method (Morse Laboratories Method #Meth-135) for determining residues of acequinocyl and acequinocyl-OH in/on nutmeats and almonds hulls; and an HPLC/MS/MS method (Morse Laboratories Method #Meth-139, Revision #2) for determining residues of acequinocyl and acequinocyl-OH in milk, meat, meat-by-products, and fat.

Based on the available method validation data (discussed below), these methods are adequate for collecting residue data in/on pome and citrus fruit commodities, tree nuts, and milk and livestock commodities. The validated LOQ for both acequinocyl and acequinocyl-OH is 0.01 ppm in/on each plant and livestock commodity, with the exception of citrus oil. The LOQ for each analyte in citrus oil is 0.5 ppm.

Methods #Meth-135 and #Meth-133, Revision #3 have each undergone successful ILV trials (MRIDs 45651602 and 45782301, respectively). An ILV was not submitted by the petitioner for Method #Meth-139, Revision #2; however, because of the similarity of all three methods, the aforementioned ILVs should be sufficient to cover this method. Methods #Meth-135, #Meth-133, Revision #3, and Method #Meth-139, Revision #2 were forwarded to the ACB for a PMV (Memo, S. Levy, 19-NOV–2003; D296183).

None of these methods were radiovalidated using samples from the submitted plant or goat metabolism studies. As the analytical method for fruits (#Meth-133) used the same solvent extraction as the apple and orange metabolism studies, radiolabeled method validation data are not required for this method. After personal communication with ACB (18-FEB-2004; C. Stafford to S. Levy), ACB determined the above HPLC/MS/MS methods only have one transition (one ion to one ion); therefore, these methods cannot serve as confirmatory methods. **Additional confirmatory methods for plants and livestock and specificity testing of the analytical enforcement methods for plants and livestock are required.**

45651604.der1

In conjunction with the apple and orange field trials, method validation trials were conducted using an HPLC/MS/MS method (Morse Method #Meth-133, Revision 3) for determining residues of acequinocyl and its metabolite, acequinocyl-OH, in/on apple and orange matrices.

For this method, residues are extracted by homogenizing with ACN or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix, or with hexane (citrus oil and dehydrated pulp). Residues are then cleaned up by ACN:hexane partitioning, gel-permeation chromatography (GPC) (dehydrate pulp only), and using silica gel solid-phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on all matrices except citrus oil, which has a LOQ of 0.5 ppm for each analyte. The limit of detection (LOD) for all analytes in/on all matrices was not reported.

Recoveries from apple fruit, juice, and wet pomace samples fortified with each analyte at 0.01 or 0.5 ppm averaged 84-96% for acequinocyl and 76-81% for acequinocyl-OH. Recoveries from orange fruit, juice, and dried pulp fortified at 0.01 or 0.5 ppm averaged 80-101% for acequinocyl and 76-99% for acequinocyl-OH, and recoveries from citrus oil at 0.5 or 25 ppm averaged 98 and 99% for acequinocyl and acequinocyl-OH, respectively.

45651609.der1

In conjunction with the almond field trials, a method validation trial was conducted using an HPLC/MS/MS method (Morse Method #Meth-135) for determining residues of acequinocyl and its metabolite, acequinocyl-OH, in/on almond hulls and nutmeats. For this method, residues are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate. Residues are then purified by solvent partitioning, GPC, and silica gel SPE. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid) with either a C18 (parent) or phenyl-hexyl (metabolite) column. Residues are detected and quantified by MS/MS detection in the positive ion mode by monitoring the transition of m/z 385 to 189 for parent and the transition of m/z 343

to 189 for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on both nutmeats and hulls. The LOD for all analytes in/on all matrices was not reported.

Method validation recoveries from samples fortified with each analyte at 0.01 or 0.05 ppm averaged 90 ± 7% for acequinocyl and 92 ± 8% for acequinocyl-OH from almond nutmeats and 90 ± 12% for acequinocyl and 95 ± 5% for acequinocyl-OH from almond hulls.

45651610.der1

In conjunction with the cattle feeding study, a method validation trial was conducted on an HPLC/MS/MS method (Morse Laboratories Method #Meth-139, Revision #2) for determining residues of acequinocyl and its metabolite, acequinocyl-OH, in/on livestock commodities. For this method, residues are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate, purified by solvent partitioning, GPC) and cleanup using silica gel SPE cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid) with a C_{18} column. Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on all livestock matrices. The LOD for all analytes in/on all matrices was not reported.

The HPLC/MS/MS Method (#Meth-139, Revision #2) is adequate for determining residues of acequinocyl and its metabolite, acequinocyl-OH, in milk and livestock commodities. For samples (n=10) fortified with acequinocyl at 0.01 or 0.1 ppm, recoveries averaged 102% from milk, 95% from muscle, 90% from liver, 107% from kidney, and 83% from fat. For samples (n=10) fortified with acequinocyl-OH at 0.01 or 0.1 ppm, recoveries averaged 93% from milk, 101% from muscle, 105% from liver, 100% from kidney, and 87% from fat.

860.1360 Multiresidue Methods

45651603.der

Acceptable Multiresidue Method testing data have been submitted for acequinocyl and acequinocyl-OH, and these data were forwarded to the U.S. FDA for further evaluation (Memo, S. Levy, 18-FEB-2004; D260953).

In summary, acequinocyl could be adequately recovered (69-82%) from apples fortified at 0.1 and 0.5 ppm using Method 302, E1 + C1 with gas chromatography (GC)/electron-capture detector (ECD), and acequinocyl-OH could be completely recovered (80-111%) from fortified (0.05 and 0.5 ppm) samples of apples and almonds using Method 402, E1 or E2 + C1 with GC/ECD. Acequinocyl-OH could not be recovered through Method 302, and neither compound could be recovered through Method 303 or 304. Acequinocyl and acequinocyl-OH were not tested through Protocols A or G as neither compound is a N-methylcarbamate or substituted urea, and acequinocyl was not tested through Protocol B as it does no have an acid or phenol moiety.

860.1380 Storage Stability

In the crop field trials, total frozen (-20 C) sample storage intervals were 77-144 days for apples, 107-144 days for pears, 59-104 days for almond nutmeats and hulls, 25-61 days for lemons, 15-110 days for oranges, and 52-111 days for grapefruit. In the apple processing study, whole fruit, wet pomace, and juice samples were stored at -20 C for 85-92 days prior to analysis. In the orange processing study, whole fruit, juice, dried pulp and oil were stored at -20 C for 10-56 days prior to analysis. The sample storage intervals from the apple and pear field trials and the apple processing study are supported by the available storage stability data. In the cattle feeding study, frozen (-20 C) storage intervals were 119-242 days for milk samples and 168-252 days for tissue samples.

The available plant storage stability data indicate that acequinocyl and acequinocyl-OH are relatively stable in frozen (-20 C) apple commodities for at least 5 months, in frozen orange RACs for at least 5 months, orange processed commodities for at least 3 months, and almond commodities for at least 3.5 months.

The available livestock storage stability study is inadequate. A new livestock storage stability study should be submitted. Day-0 samples were not analyzed; therefore, a determination of residue levels present at the time samples were placed in frozen storage could not be made. An insufficient number of time points were analyzed in order to establish that residues of acequinocyl or acequinocyl-OH were stable throughout duration of the study or to show how much of the residue was lost at various time points.

45651604.der2

Control samples of apple whole fruit, juice, and wet pomace were fortified with acequinocyl or acequinocyl-OH, each at 1.0 ppm and stored frozen for up to 5 months, with analyses at 0, 1, 3, and 5 months. The storage stability data indicate that acequinocyl and acequinocyl-OH are stable in frozen (-20 C) apple fruit, juice, and wet pomace for up to 5 months.

45651606.der1

Control samples of orange whole fruit, juice, dried pulp, and citrus oil were fortified separately with acequinocyl and acequinocyl-OH at 0.5 ppm or 25 ppm (oil only). Samples were stored frozen (-20 C). Based on analyses from the single storage interval (3 or 5 months), residues of acequinocyl and acequinocyl-OH do not appear to be stable in frozen orange matrices. After 5 months of frozen storage, acequinocyl was stable in whole oranges, but acequinocyl-OH had a decline of 23%. After 3 months of frozen storage, declines of both analytes were noted in juice (14-20%), dried pulp (27-32%), and oil (17-18%). Residue declines are comparable to declines of 3-9% per month.

45651609.der2

Control samples of almond nutmeats and hulls were fortified with acequinocyl or acequinocyl-OH, each at 1.0 ppm and stored frozen (- 20 C). Based on analyses from the single storage interval (~3.5 months), residues of acequinocyl and acequinocyl-OH do not appear to be stable in frozen almond matrices. After ~3.5 months of frozen storage, acequinocyl residues declined 20% in almond nutmeats and 14% in almond hulls, and acequinocyl-OH residues declined 24% in almond nutmeats and 20% in almond hulls. Residue declines were comparable to 4-7% per month.

45782302.der

Samples of milk, liver, kidney, muscle, and fat were spiked with acequinocyl, or its metabolite, acequinocyl-OH, each at a level of 0.5 ppm. The petitioner proposed that the mean (n=5) of method verification analyses performed in MRID 45651610 (fortified at 0.1 ppm) serve as zero day analyses. Samples were stored at -20 C $\pm$ 5C for a duration of 6-9 months. Due to the inadequacy of the study, it could not be determined if residues of parent or acequinocyl-OH increased or decreased in livestock tissue.

Under the conditions and parameters used in the study, the storage stability data are classified as scientifically unacceptable. A new livestock storage stability study should be submitted. As Day-0 samples were not analyzed, a determination of residue levels present at the time samples were placed in frozen storage could not be made. An insufficient number of time points were analyzed in order to establish that residues of acequinocyl or acequinocyl-OH were stable throughout duration of the study or to show how much of the residue was lost at various time points.

860.1400 Water, Fish, and Irrigated Crops

As there are no aquatic uses being proposed for acequinocyl, this guideline requirement is not relevant to the current petition.

860.1460 Food Handling

As there are no food handling uses being proposed for acequinocyl, this guideline requirement is not relevant to the current petition.

860.1480 Meat, Milk, Poultry, and Eggs

As none of the uses being proposed for acequinocyl include crops having regulated poultry or hog feed items, poultry and hog feeding studies are not required for this petition.

The submitted cattle feeding study is adequate, pending submission of an adequate livestock storage stability study. The conclusions discussed below are tentative and could be reevaluated based on the required storage stability data.

The MTDB for beef and dairy cattle are calculated below in Table 2. Based on a diet including wet apple pomace and almond hulls at 40 and 10%, the MTBD is 0.72 ppm for dairy cattle and 1.22 ppm for beef cattle. Based on these MTDBs, the dose levels in the cow feeding study (5, 15, and 50 ppm) are equivalent to 6.9x, 21x, and 69x the MTDB for dairy cattle and 4.1x, 12x, and 41x the MTDB for beef cattle.

Table 2. Calculation of MTDBs of Livestock for Acequinocyl.

Feed Commodity	% Dry Matter [1]	% Diet [1]	Proposed or Recommended Tolerances (ppm)	Potential Dietary Contribution (ppm) [2]
Beef and Dairy Cattle				
Wet apple pomace	40	40/20 [3]	1.0	1.0/0.5 [3]
Almond hulls	90	10	2.0	0.22
Citrus dried pulp	91	20	0.2	0.04 [4]
TOTAL BURDEN				1.22/0.72 [3]

1 Table 1 (August 1996).

2 Contribution = [tolerance / % DM (if cattle)] X % diet.

3 % diet or dietary burden for beef/ dairy cattle, respectively.

4 Dried citrus pulp was not included in dietary burden as the feeding of both wet apple pomace and dried citrus pulp is unlikely to occur.

As the combined acequinocyl residues in milk were below the combined LOQ (<0.02 ppm) in all samples from all three dose groups (up to 69x the MTDB for dairy cattle), there is no reasonable expectation of finding quantifiable acequinocyl residues in milk [40 CFR 180.6(a)(3)]. Similarly, combined residues were <LOQ in muscle from all three dose groups (up to 41x the MTDB for beef cattle) and in kidney from the 4.1 and 12x dose groups. Therefore, tolerances will not be required for milk, meat, and kidney of ruminants in this petition.

However, quantifiable residues of at least acequinocyl-OH were found in liver and fat samples from the 4.1x and 12x dose groups; therefore tolerances are required for these commodities. Considering the maximum combined residues observed in liver (<0.032 ppm) and fat (<0.013 ppm) from the 5 ppm dose group (4.1x MTDB), the maximum expected residues in liver and fat resulting from 1x the MTDB would be below the combined LOQ. Accordingly, the tolerances for residues in these commodities should be set at the LOQ (0.02 ppm) for the analytical method.

In addition to tolerances for liver and fat of cattle, tolerances should be established for liver and fat of goat, horse, and sheep.

45651610.der2.wpd

In a ruminant feeding study, three groups of lactating dairy cows (4/group) were dosed orally twice a day for 28 consecutive days with gelatin capsules containing acequinocyl at target doses equivalent to 5, 15, and 50 ppm (5 dose group (DG), 15 DG, and 50 DG) in the feed on a dry weight basis. Based on the average daily dietary intake, the actual dose levels were equivalent to 4.9, 14.9, and 48.6 ppm of acequinocyl in the diet. Cows were milked twice daily, and composited daily samples from Study Days 1, 4, 8, 12, 16, 20, 24, and 28 were collected for analysis. Cows were sacrificed within 24 hours of the final dose, and samples of muscle, liver, kidney and fat samples were collected and stored frozen until analysis. The total frozen (ca. -20 C) storage intervals were 119-242 days for whole milk and 168-252 days for all tissues. The ruminant feeding study is considered acceptable, pending submission of an acceptable storage stability study.

Milk and tissue samples were analyzed for acequinocyl and acequinocyl-OH (converted to acequinocyl-equivalents) residues using an adequate HPLC/MS/MS method (Meth-139). Briefly, residues are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate, purified using solvent partitioning, GPC, and silica SPE cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC and quantified by MS/MS detection in the positive ion mode. The LOQ for combined acequinocyl residues is 0.02 ppm in milk and all tissues. The LODs for both analytes were not reported. However, the reviewers have estimated the LODs for each analyte to be 0.001 ppm in milk, 0.002 ppm in muscle and fat, and 0.005 ppm in liver and kidneys.

With the exception of two fat samples, residues of acequinocyl were <LOD in all samples of milk and tissues from all three dose groups; whereas, residues of acequinocyl above the LOD were found in samples of milk and tissues from each dose group, with the exceptions of kidney and muscle samples from the low dose group, which had residues <LOD.

In milk, the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) were <0.002-0.003 ppm for the 5 ppm DG, <0.001-<0.005 ppm for the 15 ppm DG, and <0.003-0.013 ppm for the 50 ppm DG. Residues appeared to plateau by Study Day 4. Combined residues were <LOQ in all milk samples.

In liver, combined residues were <0.014-<0.032 ppm for the 5 ppm DG, <0.022-<0.037 ppm for the 15 ppm DG, and <0.059-<0.089 ppm for the 50 ppm DG. In kidneys, combined residues were <0.01 ppm for the 5 ppm DG, <0.010-<0.014 ppm for the 15 ppm DG, and <0.019-<0.040 ppm for the 50 ppm DG. In muscle, combined residues were <0.004 ppm for the 5 ppm DG, <0.004-<0.006 ppm for the 15 ppm DG, and <0.007-<0.008 ppm for the 50 ppm DG. In fat, combined residues in fat were <0.006-<0.013 ppm for the 5 ppm DG, <0.018-<0.030 ppm for the 15 ppm DG, and <0.044-<0.113 ppm for the 50 ppm DG.

For the 5, 15 and 50 DGs, the average combined residues were respectively <0.002, <0.003, and <0.006 ppm in milk; <0.023, <0.030, and <0.070 ppm in liver; <0.010, <0.012, and <0.034 ppm in kidneys; <0.004, <0.005, and <0.007 ppm in muscle; and <0.009, <0.022, and <0.075 ppm fat. The combined acequinocyl residues increased linearly with increased feeding level in liver and fat, but there was no linear relationship between feeding level and residues in the other tissues and milk.

Considering only the LOQ (0.01 ppm) for the two analytes, the combined residues were <LOQ in milk and muscle samples from all three dose groups and in kidney samples from the 5 and 15 ppm DG. Residues above the LOQ were found in liver and fat samples from each dose group and in kidney from the 50 ppm DG.

860.1500 Crop Field Trials

The submitted pome fruit, citrus fruit, almond, and strawberry field trial data reflect the proposed use patterns and are adequate. Details of the field trials are discussed below. The available, respective, residue data support tolerances for the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) in/on pome fruits at 0.40 ppm, citrus fruits at 0.20 ppm, almond, nutmeats at 0.02 ppm, almond, hulls at 2.0 ppm, and strawberry at 0.40 ppm.

Almonds

45651609.der3

Table 3. Summary of Residue Data for Almond Nutmeats and Hulls from Crop Field Trials with Acequinocyl (1.25 lb/gal FlC).

Almond Matrix	Total Rate (lb a.i./A) [1]	PTI [2] (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [3]	Mean	Std. Dev.
Acequinocyl Residues								
Nutmeats	0.597-0.603	7	10	<0.01 [4]	<0.01	<0.01	0.01	NA
Hulls	0.597-0.603	7	10	0.398	1.22	1.08	0.653	0.253
Acequinocyl-OH Residues								
Nutmeats	0.597-0.603	7	10	<0.01 [4]	<0.01	<0.01	0.01	NA
Hulls	0.597-0.603	7	10	0.029	0.060	0.054	0.041	0.009
Combined Residues [5]								
Nutmeats	0.597-0.603	7	10	<0.015	<0.015	<0.015	<0.015	NA
Hulls	0.597-0.603	7	10	0.436	1.29	1.14	0.699	0.262

1 The proposed maximum use rate is for two applications at 0.3 lb ai/A, for a total of 0.6 lb ai/A/season.
2 PTI = post-treatment interval; the proposed pre-harvest interval (PHI) is 7 days.
3 HAFT = Highest Average Field Trial
4 The LOQ for each analyte is 0.01 ppm in/on almond nutmeats and hulls.
5 The combined residues are expressed as acequinocyl equivalents. For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

In a total of 5 field trials conducted in CA during 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to almond trees at 0.297-0.303 lb ai/A/application, for a total of 0.599-0.603 lb ai/A/season (1x the maximum proposed rate). The applications were made during nut sizing and hull split with a 21-day RTI. In four trials, duplicate almond nutmeat and hull samples were collected at the proposed PHI, 7 days after the last application. In one residue decline trial, duplicate samples of each matrix were collected at 0, 7, 21, and 35 days post-treatment. The number of crop field trials and geographic representation of the residue data on almonds are adequate.

Almond nutmeat and hull samples were stored frozen for a maximum of 90 days (nutmeat) or 104 days (hulls), prior to analysis. The submitted stability data for acequinocyl and acequinocyl-OH residues in/on almonds and hulls samples stored frozen for up to 104 days are adequate.

Residues of acequinocyl and acequinocyl-OH in/on almond nutmeats and hulls were determined using an adequate HPLC/MS/MS method (Morse #Meth-135). The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on both almond matrices.

Following two late-season foliar applications of the acequinocyl (FlC) totaling 0.597-0.603 lb ai/A, combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) were <0.015 ppm (<combined LOQ) in/on all 10 almond nutmeat samples harvested 7 days post-treatment. Combined acequinocyl residues were also <0.015 ppm (<LOQ) in/on all 10 almond nutmeat samples harvested 0, 7, 21, and 35 days post-treatment. Combined acequinocyl residues were 0.436-1.29 ppm in/on 10 almond hull samples harvested 7 days post-treatment. Average combined acequinocyl residues in/on almond hull samples declined from 1.3 ppm at 0 days post-treatment to 0.298 ppm at 35 days post-treatment.

Citrus Fruit Group

45651606.der2

Table 4. Summary of Residue Data for Orange Fruit from Crop Field Trials with Acequinocyl (FlC).

Orange Matrix	Total Rate [1] (lb a.i./A)	PTI [2] (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [3]	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.60-0.62	7	24	0.013	0.168	0.163	0.066	0.042
Acequinocyl-OH Residues								
Fruit	0.60-0.62	7	24	<0.01 [4]	0.012	0.012	0.010	0.002
Combined Residues [5]								
Fruit	0.60-0.62	7	24	0.019	0.178	0.174	0.073	0.043

1 The proposed maximum use rate is for two applications at 0.3 lb ai/A, for a total of 0.6 lb ai/A/season.
2 PTI = post-treatment interval; the proposed PHI is 7 days.
3 HAFT = Highest Average Field Trial
4 The LOQ for each analyte is 0.01 ppm in/on orange.
5 The combined residues are expressed as acequinocyl equivalents. For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

In a total of 12 orange field trials conducted in the U.S. during 2000 and 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to orange trees at 0.30-0.31 lb ai/A/application, for a total of 0.60-0.62 lb ai/A/season (1x the maximum proposed rate). The applications were made during the later stages of fruit development with a 21-day RTI. The number of crop field trials and geographic representation of the residue data on orange are adequate. Duplicate orange fruit samples were collected at 7 days (proposed PHI) after the last application in eleven trials and at 0, 7, 21, 35, and 49 days post-treatment in the residue decline trial.

Orange fruit samples were stored frozen for a maximum of 110 days prior to analysis. The submitted stability data for acequinocyl and acequinocyl-OH in orange fruit stored frozen for up to 154 days are adequate. Residues of acequinocyl and acequinocyl-OH in/on orange fruit were determined using an adequate HPLC/MS/MS method (Morse #Meth-133, Revision 3). The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on orange fruit.

Following two applications of acequinocyl (FlC) totaling 0.60-0.62 lb ai/A (1x), combined residues of acequinocyl and acequinocyl-OH (expressed as parent equivalents) were 0.019-0.179 ppm in/on 24 orange fruit samples harvested 7 days post-treatment. Average combined acequinocyl residues in/on orange fruit samples declined from 0.106 ppm at 0 days post-treatment to 0.014 ppm at 49 days post-treatment.

45651607.der

Table 5. Summary of Residue Data for Grapefruits from Crop Field Trials with Acequinocyl (1.25 lb/gal FlC).

Grapefruit Matrix	Total Rate [1] (lb a.i./A)	PTI [2] (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [3]	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.60	6-7 [2]	12	0.021	0.077	0.071	0.044	0.018
Acequinocyl-OH Residues								
Fruit	0.60	6-7	12	<0.01 [4]	<0.01	<0.01	<0.01	NA
Combined Residues [5]								
Fruit	0.60	6-7	12	0.027	0.083	0.077	0.050	0.018

1 The proposed maximum use rate is for two applications at 0.3 lb ai/A, for a total of 0.6 lb ai/A/season.
2 PTI = post-treatment interval; the proposed PHI is 7 days.
3 HAFT = Highest Average Field Trial
4 The LOQ for each analyte is 0.01 ppm in/on grapefruit.
5 The combined residues are expressed as acequinocyl equivalents. For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

In a total of 6 grapefruit field trials conducted in the U.S. during 2000 and 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to grapefruit trees at 0.30 lb ai/A/application, for a total of 0.60 lb ai/A/season (1x the maximum proposed rate). The applications were made during the later stages of fruit development with a 21-day RTI. The number of crop field trials and geographic representation of the residue data on grapefruit are

adequate. Duplicate grapefruit fruit samples were collected at 6 or 7 days after the last application in five trials and at 0, 7, 21, 35, and 49 days post-treatment in the residue decline trial.

Grapefruit samples were stored frozen for a maximum of 111 days prior to analysis. The submitted stability data for acequinocyl and acequinocyl-OH in orange fruit stored frozen for up to 154 days are adequate. Residues of acequinocyl and acequinocyl-OH in/on grapefruits were determined using an adequate HPLC/MS/MS method (Morse #Meth-133, Revision 3). The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on grapefruits.

Following two applications of acequinocyl (FlC) totaling 0.60 lb ai/A (1x), combined residues of acequinocyl and acequinocyl-OH (expressed as parent equivalents) were 0.027-0.083 ppm in/on 12 grapefruit fruit samples harvested at the proposed PHI, ~7 days post-treatment. Average combined acequinocyl residues in/on grapefruit fruit samples declined from 0.142 ppm at 0 days post-treatment to 0.023 ppm at 49 days post-treatment.

45651608.der

Table 6. Summary of Residue Data for Lemon Fruit from Crop Field Trials with Acequinocyl (FlC).

Lemon Matrix	Total Rate [1] (lb a.i./A)	PTI [2] (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [3]	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.598-0.603	7	10	<0.01 [4]	0.079	0.079	0.046	0.028
Acequinocyl-OH Residues								
Fruit	0.598-0.603	7	10	<0.01 [4]	0.030	0.028	0.019	0.009
Combined Residues [5]								
Fruit	0.598-0.603	7	10	0.011	0.091	0.091	0.067	0.031

1 The proposed maximum use rate is for two applications at 0.3 lb ai/A, for a total of 0.6 lb ai/A/season.
2 PTI = post-treatment interval; the proposed PHI is 7 days.
3 HAFT = highest average field trial
4 The LOQ for each analyte is 0.01 ppm in/on lemons.
5 The combined residues are expressed as acequinocyl equivalents. For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

In a total of 5 lemon field trials conducted in the U.S. during 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to lemon trees at 0.299-0.302 lb ai/A/application, for a total of 0.598-0.603 lb ai/A/season (1x the maximum proposed rate). The applications were made during the later stages of fruit development with a 21-day RTI. The number of crop field trials and geographic representation of the residue data on lemon are adequate. Duplicate lemon fruit samples were collected at 7 days (proposed PHI) after the last application in four trials and at 0, 7, 21, 35, and 49 days post-treatment in the residue decline trial.

Lemon fruit samples were stored frozen for a maximum of 61 days prior to analysis. The submitted stability data for acequinocyl and acequinocyl-OH in orange fruit stored frozen for up

to 154 days are adequate. Residues of acequinocyl and acequinocyl-OH in/on lemons were determined using an adequate HPLC/MS/MS method (Morse #Meth-133, Revision 3). The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on lemon fruit.

Following two applications of acequinocyl (FlC) totaling 0.598-0.603 lb ai/A (1x), combined residues of acequinocyl and acequinocyl-OH (expressed as parent equivalents) were 0.011-0.091 ppm in/on 10 lemon fruit samples harvested 7 days post-treatment. Average combined acequinocyl residues in/on lemon fruit samples declined from 0.282 ppm at 0 days post-treatment to 0.011 ppm at 49 days post-treatment.

Pome Fruit Crop Group

45651604.der3

Table 7. Summary of Residue Data for Apple Fruits from Crop Field Trials with Acequinocyl (1.25 lb/gal FlC).

Apple Matrix	Total Rate [1] (lb a.i./A)	PTI [2] (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [3]	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.596-0.615	13-15 [2]	24	0.019	0.220	0.207	0.085	0.058
Acequinocyl-OH Residues								
Fruit	0.596-0.615	13-15	24	<0.01 [4]	<0.01	<0.01	<0.01	0
Combined Residues [5]								
Fruit	0.596-0.615	13-15	24	0.025	0.226	0.213	0.091	0.058

1 The proposed maximum use rate is for two applications at 0.3 lb ai/A, for a total of 0.6 lb ai/A/season.
2 PTI = post-treatment interval; 20 of the 24 samples were harvested at the proposed 14-day PHI.
3 HAFT = highest average field trial
4 The LOQ for each analyte is 0.01 ppm in/on apples.
5 The combined residues are expressed as acequinocyl equivalents. For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

In a total of 12 apple field trials conducted throughout the U.S. during 2000, acequinocyl (1.25 lb/gal FlC) was applied to apple trees as two broadcast foliar applications to apple trees at 0.298-0.308 lb ai/A/application, for a total of 0.596-0.615 lb ai/A/season (1x the maximum proposed rate). The applications were made during the later stages of fruit development with a 21- or 22-day RTI. The number of crop field trials and geographic representation of the residue data on apples are adequate. Duplicate samples of apple fruit were collected at 13-15 days (proposed PHI is 14 days) after the last application in eleven trials and at 0, 7, 14, and 21 days post-treatment in one residue decline trial.

Apple fruit samples were stored frozen for a maximum of 144 days prior to analysis; an interval that is supported by available stability data on apples. Residues of acequinocyl and acequinocyl-OH in/on apples were determined using an adequate HPLC/MS/MS method (Morse #Meth-133, Revision 3). The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on apples.

Following two applications of acequinocyl (FlC) totaling 0.596-0.615 lb ai/A (1x), combined residues of acequinocyl and acequinocyl-OH (expressed as parent equivalents) were 0.025-0.226 ppm in/on 24 apple fruit samples harvested 13-15 days post-treatment. Average combined acequinocyl residues in/on apple fruit samples declined from 0.226 ppm at 0 days post-treatment to 0.118 ppm at 21 days post-treatment.

45651605.der

Table 8. Summary of Residue Data for Pear Fruits from Crop Field Trials with Acequinocyl (1.25 lb/gal FlC).

Pear Matrix	Total Rate [1] (lb a.i./A)	PTI [2] (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [3]	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.591-0.610	14	12	<0.01 [4]	0.043	0.040	0.022	0.013
Acequinocyl-OH Residues								
Fruit	0.591-0.610	14	12	<0.01 [4]	<0.01	<0.01	0.01	NA
Combined Residues [5]								
Fruit	0.591-0.610	14	12	0.011	0.049	0.046	0.028	0.013

1 The proposed maximum use rate is for two applications at 0.3 lb ai/A, for a total of 0.6 lb ai/A/season.
2 PTI = post-treatment interval; the proposed PHI is 14 days.
3 HAFT = highest average field trial
4 The LOQ for each analyte is 0.01 ppm in/on pears.
5 The combined residues are expressed as acequinocyl equivalents. For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

In a total of 6 pear field trials conducted in the U.S. during 2000, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to pear trees at 0.292-0.306 lb ai/A/application, for a total of 0.591-0.610 lb ai/A/season (1x the maximum proposed rate). The applications were made during the later stages of fruit development at a 21- or 22-day RTI. The number of crop field trials and geographic representation of the residue data on pear are adequate. Duplicate pear fruit samples were collected at 14 days (proposed PHI) after the last application in five trials and at 0, 7, 14, and 21 days post-treatment in the residue decline trial.

Pear fruit samples were stored frozen for a maximum of 144 days prior to analysis; an interval that is supported by available stability data on apples. Residues of acequinocyl and acequinocyl-OH in/on pears were determined using an adequate HPLC/MS/MS method (Morse #Meth-133, Revision 2). The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on pear fruit.

Following two applications of acequinocyl (FlC) totaling 0.591-0.610 lb ai/A, combined residues of acequinocyl and acequinocyl-OH (expressed as parent equivalents) were 0.011-0.049 ppm in/on 12 pear fruit samples harvested 14 days post-treatment. Average combined acequinocyl residues in/on pear fruit samples declined from 0.164 ppm at 0 days post-treatment to 0.030 ppm at 21 days post-treatment.

Strawberry

45896801.der

Table 9. Summary of Residue Data for Strawberry from Crop Field Trials with Acequinocyl (1.25 lb/gal FlC).

Strawberry Matrix	Total Applic. Rate, (lb a.i./A)	PHI[1] (days)	Residue Levels (ppm)					
			n	Min.	Max.	HAFT[2]	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.30	1	16	0.140	0.345	0.343	0.222	0.063
Acequinocyl-OH Residues								
Fruit	0.30	1	16	<0.01[3]	0.0118	0.0084	<0.01	0.002
Combined Residues[4]								
Fruit	0.30	1	16	0.145	0.350	0.348	0.227	0.064

1 The proposed PHI is 1 day.
2 HAFT = Highest Average Field Trial.
3 The LOQ for both analytes is 0.01 ppm in/on strawberries.
4 For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

In a total of 8 strawberry field trials conducted in the U.S. during the 2002 growing season, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to strawberries at 0.292-0.304 lb ai/A/application, for a total of 0.588-0.603 lb ai/A/season (1x the maximum proposed rate). The applications were made at 22 days and 1 day before harvest. The number of crop field trials and geographic representation of the residue data on strawberries are adequate. Duplicate strawberry fruit samples were collected at 1 day (proposed PHI) after the last application in seven trials and at 0, 1, 4, 7, and 14 days post-treatment in the residue decline trial.

Strawberry fruit samples were stored frozen for a maximum of 110 days prior to analysis, an interval that is supported by available stability data on apples. Strawberry matrices were analyzed for residues of acequinocyl and acequinocyl-OH using an adequate HPLC/MS/MS method (Morse #Meth-133, Revision 3). The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on strawberry fruit.

The results from these trials show that combined residues of acequinocyl and acequinocyl-OH were 0.145-0.350 ppm in/on 16 strawberry fruit samples harvested 1 day post-treatment. Average combined acequinocyl residues in/on strawberry samples declined from 0.427 ppm at 0 days post-treatment to 0.022 ppm at 14 days post-treatment. Residue decline data show that acequinocyl residues appear to decrease in strawberries with increasing PHIs.

860.1520 Processed Food and Feed

An adequate processing study was submitted to support the proposed use on pome fruits. As the processing factor for apple juice (0.03x) was ≤1x, a tolerance is not required for apple juice. However, residue concentrations were observed in wet apple pomace (3.5x). Based on the observed 3.5x processing factor for wet apple pomace and HAFT residues of 0.213 ppm from the apple field trials, the maximum expected acequinocyl residues in wet apple pomace would be 0.75 ppm. These data support a tolerance of 1.0 ppm for residues in wet apple pomace.

An adequate processing study was submitted to support the proposed use on citrus fruits. The study indicates that combined residues of acequinocyl did not concentrate appreciably in orange juice (0.04x) or dried pulp (1.09x). Therefore, separate tolerances would not be required for these commodities. However, residue concentrations were observed in citrus oil (165x). Based on the 165x processing factor for oil and HAFT residues of 0.174 ppm from the apple field trials, the maximum expected acequinocyl residues in citrus oil would be 28.7 ppm. These data support a tolerance of 30 ppm for residues in citrus oil.

Apple

45651604.der4

In one trial conducted in NY, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to apple trees at 1.21-1.22 lb ai/A/application, for a total of 2.43 lb ai/A/season (4.1x the maximum proposed rate). Applications were made during the later stages of fruit development. Apple fruit samples were collected at 14 days after the last application and subsamples were processed into wet pomace and juice using simulated commercial procedures.

Samples were stored frozen for a maximum of 92 days, an interval supported by available stability data. Apple matrices were analyzed for residues of acequinocyl and acequinocyl-OH using an adequate HPLC/MS/MS method (Morse #Meth-133, Revision 3), which has an LOQ 0.01 ppm for parent and acequinocyl-OH in each matrix.

Combined residues of acequinocyl and acequinocyl-OH (expressed as parent equivalents) were 0.337 and 0.356 ppm in/on 2 apple fruit samples, 1.19 and 1.27 ppm in 2 wet pomace samples, and <0.01 ppm in 2 juice samples. The average processing factors of combined acequinocyl residues were 3.5x in wet pomace and 0.03x in juice. The maximum theoretical concentration factor for apples is >14x.

Orange

45651606.der3

In a trial conducted in CA, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to orange trees in the later stages of fruit development at 1.18 lb ai/A/application, for a total of 2.36 lb ai/A/season (4x the maximum proposed rate). Orange fruits were sampled at 7 days after the last application and subsamples were processed into dried pulp, juice, and oil using simulated commercial procedures.

Samples were stored frozen for a maximum of 56 days, an interval supported by the available stability data. Orange matrices were analyzed for residues of acequinocyl and acequinocyl-OH using an adequate HPLC/MS/MS method (Morse #Meth-133, Revision 3), which has an LOQ of 0.01 ppm for each analyte in each matrix except citrus oil, which has a LOQ of 0.5 ppm.

Combined residues of acequinocyl and acequinocyl-OH (expressed as parent equivalents) were 0.293 and 0.241 ppm in/on 2 orange fruit samples, 0.318 and 0.263 ppm in 2 dried pulp samples, <0.01 ppm in 2 juice samples, and 44.5 and 43.7 ppm in 2 oil samples. The average processing factors for the combined acequinocyl residues were 0.04x in juice, 1.09x in dried pulp, and 165x in oil. The maximum theoretical concentration factor for citrus is 1000x.

860.1650 Submittal of Analytical Reference Standards

The analytical reference standard for acequinocyl-OH has not been submitted to the EPA National Pesticide Standards Repository (electronic communication, S. Levy and C. Stafford to T. Cole, 08-JAN-2003). **This is a deficiency.**

860.1850/1900 Confined and Field Accumulation in Rotational Crops

A confined or field accumulation study were not submitted with these petitions. As strawberries are rotated crops, a confined rotational crop study should be submitted. Until an acceptable study is submitted, rotation should be prohibited to any crop other than strawberries. **A revised Section B should be submitted.**

860.1550 Proposed Tolerances

There are currently no U.S. tolerances established for acequinocyl in or on food and feed commodities. The HED MARC determined that for tolerance expression, parent and acequinocyl-OH are the residues of concern in plant and ruminant commodities (Memo, S. Levy, et.al., 07-JAN-2004; TXR #: 0052294). The petitioner has proposed that tolerances for acequinocyl in/on the plant and livestock commodities listed below in Table 10. **A revised Section F should be submitted.**

The available apple and pear field trial data and the apple processing study support the proposed tolerances for the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) in/on pome fruits at 0.40 ppm and in wet apple pomace at 1.0 ppm.

The grapefruit, lemon, and orange field trial data support a tolerance for the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) of 0.20 ppm for citrus fruits, and the almond field trail data support tolerances for the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) of 0.02 ppm for almond and 2.0 for almond, hulls. In addition, the orange processing data support a tolerance for the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) of 30 ppm tolerance for citrus oil. The almond data are translated to support a tolerance for the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) of 0.02 ppm for pistachio.

Although additional storage stability data are required to upgrade the cattle feeding study to adequate, data from the feeding study suggest that tolerances will not be necessary for residues in milk, meat, and kidneys of ruminants, as there is no reasonable expectation of finding quantifiable residues in these commodities [40 CFR 180.6(a)(3)] even at level up to 10x the MTDB. However, tolerances are likely to be required for combined acequinocyl residues in fat and liver of cattle, goat, horse, and sheep. If the requested storage stability data indicate that residues are stable in frozen livestock commodities, then tolerances for the combined acequinocyl residues in liver and fat should be set at the LOQ (0.02 ppm) for the analytical method.

Tolerances for poultry and hog commodities are not required as none of the proposed uses include crops having poultry or hog feed items.

Table 10. Tolerance Summary for Acequinocyl.

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments *Correct commodity definition*
Almond, nutmeat	0.01	0.02	Tolerance is based on combined LOQ for each analyte. *Almond*
Almond, hulls	1.5	2.0	
Apple, wet pomace	1.0	1.0	
Citrus Fruit Crop Group	0.3	0.20	*Fruit, citrus, group 10*
Cow, fat	0.02	0.02 [1]	Separate tolerances are also required for fat of goat, horse, and sheep. *Cattle, fat; Goat, fat; Horse, fat, and Sheep, fat*
Cow, kidney	0.01	Not required (NR)	situation 40 CFR 180.6(a)(3)
Cow, liver	0.02	0.02 [1]	Separate tolerances are also required for liver of goat, horse, and sheep. *Cattle, liver; Goat, liver; Horse, liver, and Sheep, liver*
Cow, muscle	0.01	NR	situation 40 CFR 180.6(a)(3)
Milk	0.01	NR	situation 40 CFR 180.6(a)(3)
Orange, oil	30	30	*Citrus, oil*
Pistachio, nutmeat	0.01	0.02	Tolerance is based on residue data for almonds. *Pistachio*
Pome Fruit Crop Group	0.4	0.40	*Fruit, pome, group 11*
Strawberries	0.4	0.40	*Strawberry*

[1] Recommended tolerances are tentative because storage stability data are required to support the cattle feeding study.

International Limits

There are no established or proposed Codex, Canadian or Mexican maximum residue limits (MRLs) for acequinocyl (see Attachment 1 (International Residue Limit Status (IRLS) Sheet)).

Attachments

Attachment 1: IRLS Sheet.
Attachment 2: Identification of Compounds.

References

45651701.der	860.1300 Nature of the Residue (Apple)
45651702.der	860.1300 Nature of the Residue (Orange)
45651703.der	860.1300 Nature of the Residue (Eggplant)
45651704.der	860.1300 Nature of the Residue (Ruminants)
45651604.der1	860.1340 Residue Analytical Methods (Crops)
45651609.der1	860.1340 Residue Analytical Methods (Crops)
45651610.der1	860.1340 Residue Analytical Methods (Livestock)
45651603.der	860.1360 Multiresidue Methods
45651604.der2	860.1380 Storage Stability (Crops)
45651606.der1	860.1380 Storage Stability (Crops)
45651609.der2	860.1380 Storage Stability (Crops)
45782302.der	860.1380 Storage Stability (Livestock)
45651610.der2	860.1480 Meat, Milk, Poultry, and Eggs (Ruminants)
45651609.der3	860.1500 Crop Field Trials (Almond)
45651606.der2	860.1500 Crop Field Trials (Citrus)
45651607.der	860.1500 Crop Field Trials (Citrus)
45651608.der	860.1500 Crop Field Trials (Citrus)
45651604.der3	860.1500 Crop Field Trials (Pome Fruit)
45651605.der	860.1500 Crop Field Trials (Pome Fruit)
45896801.der	860.1500 Crop Field Trials (Strawberry)
45651604.der4	860.1520 Processed Food and Feed (Apple)
45651606.der3	860.1520 Processed Food and Feed (Orange)

cc: S. Levy (RAB1)
RDI: Chem SAC (14-JAN-2004), P.V. Shah (28-APR-2004), RAB1 Chemists (10-DEC-2003)
S. J. Levy:806T:CM#2:(703)305-0783:7509C:RAB1

Attachment 1: IRLS Sheet.

INTERNATIONAL RESIDUE LIMIT STATUS

Chemical Name: 2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione	Common Name: Acequinocyl	X Proposed tolerance ☐ Reevaluated tolerance ☐ Other	Date: 15-DEC-2003

Codex Status (Maximum Residue Limits)	U. S. Tolerances
X No Codex proposal step 6 or above ☐ No Codex proposal step 6 or above for the crops requested	Petition Numbers: PP#2F06440, 3F06595 DP Barcodes: D284757, D290204 Other Identifier:
Residue definition (step 8/CXL): N/A	Reviewer/Branch: S. Levy/RAB1
	Residue definition: Combined residues of Acequinocyl and Acequinocyl-OH, expressed as parent

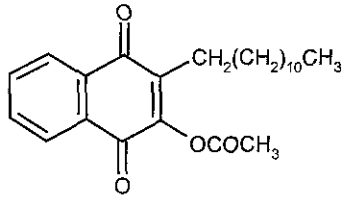
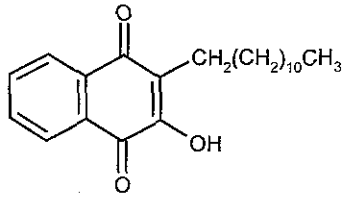
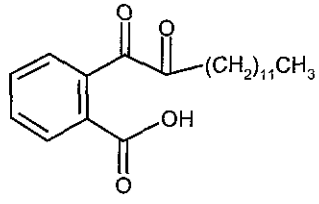
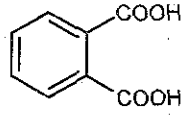
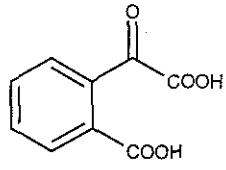
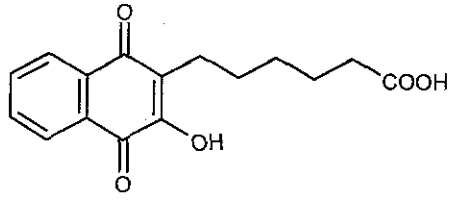
Crop (s)	MRL (mg/kg)	Crop(s)	Tolerance (ppm) [1]
		Almond, nutmeat	0.02
		Almond, hulls	2.0
		Apple, wet pomace	1.0
		Citrus Fruit Crop Group	0.20
		Cow, fat	0.02
		Cow, liver	0.02
		Orange, oil	30
		Pistachio, nutmeat	0.02
		Pome Fruit Crop Group	0.40
		Strawberry	0.40

Limits for Canada		Limits for Mexico	
X No Limits ☐ No Limits for the crops requested		X No Limits ☐ No Limits for the crops requested	
Residue definition: N/A		Residue definition: N/A	
Crop(s)	MRL (mg/kg)	Crop(s)	MRL (mg/kg)

Notes/Special Instructions: S. Funk, 01/12/04

[1] Recommended tolerances. Tolerances of livestock commodities are tentative as additional supporting storage stability data are required.

Attachment 2: Identification of Compounds.

Common name/code	Chemical name	Chemical structure
Acequinocyl AKD-2023	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione	
Acequinocyl-OH (Metabolite R1)	2-dodecyl-3-hydroxy-1,4-naphthalenedione	
AKM-18	2-(1,2-dioxotetradecyl)-benzoic acid	
phthalic acid		
CBAA	2-carboxy-α-oxo-benzene acetic acid	
AKM-15		

Acequinocyl/PC Code 006329/Arvesta
DACO 6.3/OPPTS 860.1300/OECD II 6.2.2, 6.2.3 & IIIA 8.2, 8.4.1, 8.4.2
Nature of the Residues in Plants - Apple

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 02-APR-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651701 Mayo, B. (1997) ^{14}C-AKD 2023 Metabolism in Apples: Lab Project Number: AGK 20/950695. Unpublished study prepared by Huntingdon Life Sciences, Ltd. and Arvesta Corporation. 201 pages.

EXECUTIVE SUMMARY:

In an apple metabolism study, [U-^{14}C-phenyl]- or [1-^{14}C-dodecyl]acequinocyl (≥95% radiochemical purity) was formulated as a flowable concentrate (FlC) and applied once as a broadcast foliar application during fruit development to apple trees grown outdoors in containers. [^{14}C]Acequinocyl was applied at rates equivalent to 0.76-0.776 kg ai/ha (0.637-0.691 lb ai/A). Fruit samples were collected at 0, 14, 21, and 30 days after treatment (DAT) and leaf samples were collected at 0 and 30 DAT.

The levels of total radioactive residues (TRR) in/on apple fruits and leaves were generally similar for the two ^{14}C-labels. On the day of application, average TRRs were 1.302 and 1.386 ppm in/on fruits and 53.92 and 54.13 ppm in/on leaves from both ^{14}C-labels. Decline in TRR values were variable. TRR in/on [^{14}C-PH]-treated fruits initially declined to 0.384 ppm at 14 DAT, but was 0.584-0.592 ppm by 21 and 30 DAT. For the [^{14}C-DOD]-treated fruits, TRR declined to 0.668-0.698 ppm by 14-30 DAT. For leaves, TRR declined to 23.72 ppm in/on [^{14}C-PH]-treated leaves and to 4.6 ppm in/on [^{14}C-DOD]-treated leaves by 30 DAT. Translocation of ^{14}C-residues from leaves to fruits was minimal. When fruits were covered prior to foliar application of either ^{14}C-label, TRR in/on fruits were 0.014-0.016 ppm at 30 DAT.

For both ^{14}C-labels, the majority of the TRR consisted of surface residues. Surface ^{14}C-residues in/on fruit and leaves accounted for the 98.0-98.7% of the TRR at 0 DAT and declined to 39.9-63.2% of the TRR at 30 DAT. The percentage of the fruit TRR associated with the fruit peel and flesh increased over time. By 30 DAT, radioactivity in the peel accounted for 28.6-44.1% of the TRR and radioactivity in the flesh accounted for 8.2-10.5% of the TRR.

Surface washes and solvent extractions released 68.4-99.7% of the TRR from fruits and leaves, and base extractions with 1M and 5M NaOH at 55 C released an additional 13.8-29.5% of the TRR. The overall recovery of radioactivity from fruit and leaf samples was 98.7-100.1%. High-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) analyses identified 48.5-94.0% of the TRR in/on fruits and 35.2-93.5% of the TRR in/on leaves, with ^{14}C-residues being identified by co-chromatography with reference standards. Sufficient information was available to assess the stability of ^{14}C-residues; no additional sample storage information or stability data are required.

The metabolite profile was similar between the two ^{14}C-labels and between fruits and leaves. For fruits, acequinocyl accounted for 88.7-92.0% of the TRR at the 0 DAT and 28.4-57.0% of the TRR at 14-30 DAT. Minor amounts of Metabolite R1 (1.4-4.4% TRR) and AKM-18 (0.1-2.1% TRR) were also identified in fruits at each interval. Phthalic acid was also identified in fruits (~8.5-17.1% TRR) from the 14-30 DAT intervals, although the quantities reported are approximate as TLC separation was not complete. The metabolite 2-carboxy-α-oxo-benzene acetic acid (CBAA) was also tentatively identified in [^{14}C-PH]-labeled fruits as a minor component of the polar residues. The remaining solubilized ^{14}C-residues from fruits were comprised primarily of unknown polar metabolites, each accounting for <5% of the TRR.

For leaves, acequinocyl accounted for 91.2-93.5% of the TRR at 0 DAT and 20.0-26.0% of the TRR by 30 DAT. At both intervals, Metabolite R1 accounted for 0.9-1.5% of the TRR and AKM-18 accounted for 0.2-4.8% of the TRR. Residues of phthalic acid were also identified as a component of the polar ^{14}C-residues at 30 DAT (10.5-12.4% TRR). Unidentified, extractable ^{14}C-residues accounted for a total of 35.2-44.5% of the TRR by 30 DAT and were comprised primarily of polar metabolites, each accounting for <8% of the TRR.

Based on the identified metabolites, the metabolism of acequinocyl in apples appears to involve the loss of the acetyloxy moiety to form Metabolite R1, opening of the quinone ring to form AKM-18, and subsequent degradation of the quinone ring to yield phthalic acid.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the metabolism data on apples are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance and Data Confidentiality statements were provided.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

TABLE A.1. Test Compound Nomenclature

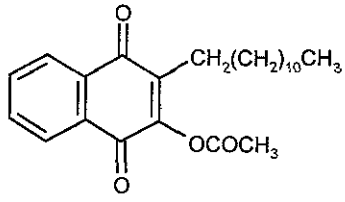
Compound	Chemical Structure
	
Common name	Acequinocyl
Company experimental name	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use product/EP	Kanemite™ 15 SC, 1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties

Parameter	Value	Reference (MRID#)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (mg/L at 20°C)	1,2-dichloroethane: >250 acetone: >250 ethyl acetate: >250 xylene: >250 methanol: 6.1 n-heptane: 36 n-octanol: 29.2	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

The in-life and analytical phases of the study were conducted by Huntingdon Life Sciences, Ltd. (Cambridgeshire, England) from 29-JUL-1994 to 20-FEB-1997. Apple trees were grown individually in containers in an outdoor netted tunnel enclosure. Plants were watered regularly and supplied periodically with liquid fertilizer and fungicides as needed. Growth and development of the plants was normal, and no problems were noted.

TABLE B.1.1. Test Site Information

Testing Environment	Soil characteristics			
	Type	%OM [1]	pH	CEC [2]
Plants were grown outdoors in individual containers in a netted tunnel enclosure	NR [3]	NR	NR	NR

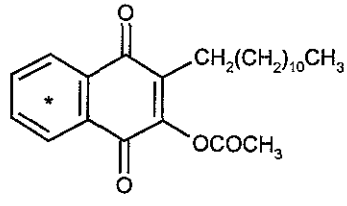
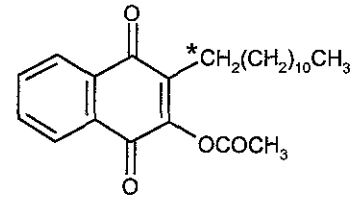
1 %OM = % organic matter.
2 CEC = cation-exchange capacity.
3 NR = not reported.

TABLE B.1.2. Crop Information

Crop/crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested RAC	Harvesting procedure
Apple (*Malus domestica*)/ Pome Fruit	Golden Delicious	Fruit development	Mature	Fruit and leaves	Hand

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics

Chemical structure	 * indicates ^{14}C-position	 * indicates ^{14}C-position
Radiolabel position	Uniformly ^{14}C-labeled in the phenyl ring	^{14}C-labeled at the C-1 position of the dodecyl side chain
Lot No.	CP-1526	CP-1525
Radiochemical Purity [1]	≥95%	≥95%
Specific activity [1]	2.28 MBq/mg (137,000 dpm/µg)	2.15 MBq/mg (129,000 dpm/µg)
Code	[^{14}C-PH]	[^{14}C-DOD]

1 The test substances were formulated as a FlC and diluted with water for application. The radiochemical purity and specific activity of formulated test substances.

B.3. Study Use Pattern

[^{14}C-PH]- and [^{14}C-DOD]-Acequinocyl, each formulated as a FlC, were applied separately to apple trees growing outdoors in pots, as a single broadcast foliar application at a rates equivalent to 716 and 776 g ai/ha, respectively (Table B.3.1). The application was made during fruit development, approximately 30 days prior to normal harvest. To examine translocation, a separate branches on selected trees were covered with plastic bags during application. To provide samples for examination of polar metabolites, a supplemental study was conducted in which apples received a single broadcast foliar application of [^{14}C-PH]- and [^{14}C-DOD]-acequinocyl at rates of 616 and 669 g ai/ha, respectively. In the main study, [^{14}C-PH]-acequinocyl was applied on 7/29/94 and the [^{14}C-DOD]-acequinocyl was applied on 8/4/94. In the supplemental study, the [^{14}C-PH]-acequinocyl was applied on 8/29/96 and the [^{14}C-DOD]-acequinocyl was applied on 8/28/96.

TABLE B.3.1. Use Pattern Information

Main Study		
Chemical name	[^{14}C-PH]Acequinocyl	[^{14}C-DOD]Acequinocyl
Application method	foliar	foliar
Application rate (kg ai/ha)	0.716 (0.637 lb ai/A)	0.776 (0.691 lb ai/A)
Number of applications	1	1
Timing of applications	Broadcast foliar application during fruit development	
PHI(s) for fruits and leaves	0, 14, 21, 30 (fruit); 0, 30 (leaves)	0, 14, 21, 30 (fruit); 0, 30 (leaves)
Supplemental Study		
Chemical	[^{14}C-PH]Acequinocyl	[^{14}C-DOD]Acequinocyl
Application method	foliar	foliar
Application rate (kg ai/ha)	0.616 (0.548 lb ai/A)	0.669 (0.595 lb ai/A)
Number of applications	1	1
Timing of applications	Broadcast foliar application during fruit development	
PHI(s) for leaves and fruits	28	28

Following the broadcast foliar application with each ^{14}C-label, 3 individual fruits were collected at 0 (<0.5 hour), 14, and 21 DAT and 10 individual fruits were collected at 30 DAT. For the leaf samples, 3 leaf samples were collected at 0 DAT and 6 leaf samples were collected at 30 DAT from each ^{14}C-label. In addition, four fruits, which were covered during application, were collected at 30 DAT. In the supplemental study, 2 or 3 batches of fruit (3/batch) and leaves (6/batch) were collected at 28 DAT. All samples were stored at <-15 C.

B.4. Identification/ Characterization of Residues

B.4.1. Sample Preparation

Immediately following collection, individual fruit and leaf samples were surface rinsed three times by sonicating in acetonitrile (ACN). Fruit samples were then divided into peel and flesh fractions. ^{14}C-Residues in the individual peel, flesh, and leaf samples were then extracted by homogenizing 3 times in ACN, centrifuging after each extraction. Extract fractions were pooled by sample and concentrated at 37 C. Surface rinse and extract fractions containing sufficient radioactivity were analyzed by reverse-phase HPLC and normal-phase TLC.

TRR in each sample were determined by summing radioactivity in surface washes, extracts, and residual solids. The data from the analysis of the individual samples were reported as averages with standard deviation. Radioactivity in liquid fractions was determined by liquid-scintillation counting (LSC) and radioactivity in residual solids was determined by combustion LSC. The limit of detection (LOD) for the radioassays was not reported.

For both ^{14}C-labels, post-extraction solid (PES) fractions accounting for >10% radioactivity from peel samples (14-30 DAT) and leaf samples (30 DAT) were subjected to further extractions. Residues were extracted with ACN:water (1:1, v/v) in a sonic bath, then centrifuged. Residual solids were then further extracted with 1M NaOH at 55 C for 18 hours, then at ambient temperature. The extraction procedure was repeated with 5M NaOH. The resulting extracts were analyzed by HPLC and TLC.

To further examine the nature of the polar ^{14}C-residues in apples, fruit samples (28 DAT) from plants treated with either ^{14}C-label at 0.616 or 0.669 kg ai/ha (0.548 or 0.595 lb ai/A) in the supplemental study were surface rinsed, separated into peel and flesh, and extracted as described above for the main study. The resulting peel extracts were analyzed by HPLC and TLC. In addition, polar ^{14}C-residues from peel extracts (30 DAT) from the main study were dried, suspended in a buffer solution, and subjected to enzymatic hydrolyses with pectinase, protease, or a mixture of cellulase, hemicellulase, β-glucosidase, and xylanase. The hydrolysates were then examined by TLC and HPLC.

B.4.2. Analytical Methodology

Radioactive residues in solvent fractions were profiled and quantified by reverse-phase HPLC and/or normal-phase 1D- and 2D-TLC. For quantifying ^{14}C-residues in solvent fractions, the HPLC systems consisted of a reverse-phase column (C_8 or PRP-1) using a mobile phase gradient of 0.015 or 0.1 M ammonium formate (pH 3.4 or 7) to ACN, with UV and radioactivity detectors. For more detailed analyses of polar components, the HPLC systems consisted of a reverse-phase column (YMC-AQ) using a mobile phase gradient of 0.01 M ammonium formate (pH 3) to ACN, with UV and radioactivity detectors. The TLC analyses used silica gel or reverse-phase plates with a variety (6) of solvent systems. Components were identified by co-chromatography with reference standards on at least two systems. In addition, isolated parent

was analyzed by mass spectral analysis.

Including parent, a total of 25 reference standards were used for comparison. Reference standards were detected by UV absorbance (254 nm) for HPLC and by UV quenching on TLC plates. Radioactivity eluting from the HPLC was quantified by LSC of collected fractions, and radioactivity on TLC plates was detected and quantified using either a Berthold linear analyzer (1D-TLC) or a Fuji BAS 2000 Bioimage Analyzer (2D-TLC).

C. RESULTS AND DISCUSSION

The methods used to conduct both the in-life and analytical phases of the apple metabolism study are adequate. Although information on the LOD for the radioassays was not reported, the specific activity of the ^{14}C-test substances and the levels of radioactivity in the harvested samples were sufficient to allow for identification and characterization of the ^{14}C-residues.

Following a single foliar application of either [^{14}C-PH] or [^{14}C-DOD]-acequinocyl (FlC) at 0.616 and 0.669 kg ai/ha (0.637 and 0.691 lb ai/A), respectively, TRR in/on apple fruits and leaves were generally similar for the two ^{14}C-labels (Table C.2.1.1). On the day of application, average TRRs were 1.386 and 1.302 ppm in/on fruits and 53.92 and 54.13 ppm in/on leaves from the [^{14}C-PH] and [^{14}C-DOD]-labels, respectively. Decline in TRR values were variable. TRR in/on [^{14}C-PH]-treated fruits initially declined to 0.384 ppm at 14 DAT, but was 0.584-0.592 ppm by 21 and 30 DAT. For the [^{14}C-DOD]-treated fruits, TRR declined and remained steady at 0.668-0.698 ppm from 14-30 DAT. For leaves, TRR declined by 30 DAT to 23.72 ppm in/on [^{14}C-PH]-treated leaves and to 4.6 ppm in/on [^{14}C-DOD]-treated leaves.

For both ^{14}C-labels, the majority of the TRR in fruit samples consisted of surface residues. Immediately following application (0 DAT), surface ^{14}C-residues on apple fruits accounted for the 98.2-98.7% of the TRR. The %TRR recovered in the surface rinse declined steadily with time. By 30 DAT, surface ^{14}C-residues on fruit accounted for the 45.4-63.2% of the TRR, and there was a concomitant increase in the percentage of the fruit TRR associated with the peel and flesh. By 30 DAT, radioactivity in the peel accounted for 28.6-44.1% of the TRR and radioactivity in the flesh accounted for 8.2-10.5% of the TRR. For leaf samples from both ^{14}C-labels, surface ^{14}C-residues on leaves accounted for the 98.0-98.5% of the TRR at 0 DAT. By 30 DAT, surface residues on the leaves declined to 39.9-48.9% of the TRR, and the majority of the TRR was recovered in the leaf (51.1-60.1% TRR).

Translocation of ^{14}C-residues from leaves to fruits was minimal. When selected branches were covered prior to foliar application of either ^{14}C-label, TRR in/on covered fruits were 0.014-0.016 ppm at 30 DAT (Table C.2.1.2), and TRR in/on covered leaves were 0.148-0.166 ppm.

The distribution and fractionation of radioactivity in/on fruits and leaves following foliar application of the two ^{14}C-labels were similar (Tables C.2.2.1-C.2.2.4). Surface rinses with ACN and solvent extractions (ACN and ACN:water) released >99% of the TRR from fruits at 0 DAT and 67.0-82.4% of the TRR from fruits at 14-30 DAT. At the 14-30 DAT intervals, peel extracts accounted for 5.9-14.1% of the TRR (0.041-0.083 ppm) and flesh extracts accounted for 2.0-7.1% of the TRR (0.003-0.041 ppm). PES from peel accounted for 15.3-32.2% of the TRR (≤0.188 ppm) and the PES fractions from flesh accounted for 3.0-5.1% of the TRR (≤0.034 ppm). Extractions of the peel PES fractions with 1 and 5M NaOH released an additional 13.8-27.5% of the TRR. The overall recovery of radioactivity from the fruit samples was 99.8-100.1%.

For leaves, surface washes and solvent extractions released 99.1-99.5% of the TRR at 0 DAT and 68.4-72.2% of the TRR at 30 DAT. Extraction of the PES fractions from 30 DAT with NaOH released an additional 27.3-29.5% of the TRR. The overall recovery of radioactivity from the leaf samples was 98.7-100.0%.

HPLC and TLC analyses of the surface rinse fractions identified 91.6-94.0% of the TRR in/on fruits at 0 DAT; acequinocyl was the major ^{14}C-residue accounting for 88.7-92.0% of the TRR along with minor amounts of Metabolite R1 (1.9-2.6% TRR) and AKM-18 (≤0.3% TRR). At the later sampling intervals (14, 21 and 30 DAT), 48.5-68.9% of the TRR in/on fruits was identified. The metabolite profile in/on fruits was similar for both ^{14}C-labels. Acequinocyl was the major ^{14}C-residue in/on fruits at all sampling intervals, accounting for 47.8-57.0% of the TRR at 14 DAT, 35.6-54.0% of the TRR by 21 DAT, and 28.4-41.2% of the TRR by 30 DAT. Minor amounts of Metabolite R1 (1.4-4.4% TRR) and AKM-18 (0.1-2.1% TRR) were also identified. Phthalic acid was also identified in fruits (8.5-17.1% TRR) from the 14-30 DAT intervals; however, the amounts are only approximate as separation by TLC was not complete. The remaining solubilized ^{14}C-residues from fruits were comprised primarily of additional polar metabolites, each accounting for <5% of the TRR. The unknown polar residues increased from 4.3-7.2% of the TRR on 0 DAT to 24.2-29.9% of the TRR by 30 DAT.

The metabolite profile in leaves was similar to fruits, although the percentage of polar metabolites was higher at the final sampling interval for leaves. Acequinocyl was the major ^{14}C-residue in/on leaves, accounting for 91.2-93.5% of the TRR at 0 DAT and 20.0-26.0% of the TRR by 30 DAT. At both intervals, Metabolite R1 accounted for 0.9-1.5% of the TRR and AKM-18 accounted for 0.2-4.8% of the TRR. Residues of phthalic acid were not quantified in leaves at 0 DAT, but were identified as a component of the polar ^{14}C-residues at 30 DAT (10.5-12.4% TRR). Unidentified, extractable ^{14}C-residues accounted for a total of 35.2-44.5% of the TRR by 30 DAT and were comprised primarily of minor polar metabolites, each accounting for <8% of the TRR.

In the additional work conducted on the unknown polar ^{14}C-residues, the polar ^{14}C-residues were separated into three major regions, 1A, 1B, and 1C. Unknown 1C was shown to consist of phthalic acid by HPLC and TLC analyses. Treatment with various hydrolytic enzymes (cellulase, hemicellulase, β-glucosidase, xylosidase, pectinase, and protease) had no appreciable affect on

the proportion of these three components. In addition, TLC and HPLC analyses of the polar fraction from peel extracts of [^{14}C-phenyl]-treated fruits suggested that 2-carboxy-α-oxo-benzene acetic acid (CBAA) may be a minor component of the polar ^{14}C-residues. Using one of the TLC systems (4b), CBAA separated into two bands, possibly indicating the presence of different ionic forms. The two bands corresponded to polar fractions 1A and 1B, which each accounted for <5% of the TRR.

C.1. Storage Stability

Samples of apple leaves and fruits from the main study were immediately surface washed after collection. All samples and sample fractions were then stored at <-15 C.

The only storage stability data provided in the report were from the repeated HPLC analyses of surface washes from fruit samples (both ^{14}C-labels) on two dates and from two TLC analyses of peel extracts on two dates. The provided HPLC chromatograms indicate that surface washes from fruits were initially profiled within ~1 month of collection. The subsequent reanalysis of these fractions 3 months later indicates that residues of parent, which was the principle component, were stable in frozen storage. The TLC analyses of the peel extracts suggest that these extracts were initially profiled after ~3 months of frozen storage. The initial analyses show parent and two polar metabolites (1A and 1B); however, the reanalysis ~6 months later shows that there was substantial loss of one of the polar residues (Unknown 1B).

Considering that (i) parent was the major ^{14}C-residue (20.0-93.5% TRR) in/on fruit and leaves; (ii) parent was apparently stable in the fruit surface washes and extracts over the course of analysis; (iii) the polar components which showed changes during storage were minor components (<5% TRR) of the ^{14}C-residue; and (iv) the additional work done on characterizing the polar residues utilized newly generated samples, no additional sample storage information or stability data will be required to support this metabolism study.

TABLE C.1. Summary of Storage Conditions

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Study Duration (months)	Limit of Demonstrated Storage Stability (days or months)
Fruits (surface rinses and peel extracts)	<-15 C	1-8 [1]	Parent was apparently stable in surface washes and extracts for ~3 months.
Fruits (flesh extracts)		Not provided	
Leaves (surface rinses and leaf extracts)		1-2	

[1] Peel extracts from 30 DAT fruit treated with the C^{14}-DOD label were stored for 8 months, all others were stored 1-2 months.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1.1 Total Radioactive Residues (TRRs) in/on Apple Fruits and Leaves Following a Single Foliar Application of [^{14}C-PH] or [^{14}C-DOD]-Acequinocyl at 0.637 and 0.691 lb ai/A, Respectively. [1]

Sample	Sampling interval (DAT)	Fraction	[^{14}C-PH]-Acequinocyl		[^{14}C-DOD]-Acequinocyl	
			% TRR	ppm	% TRR	ppm
Fruit	0	Surface wash	98.7	1.368	98.2	1.279
		Peel [2]	1.0	0.014	1.3	0.017
		Flesh [2]	0.2	0.003	0.4 [3]	0.005 [3]
		Total	NA	1.386	NA	1.302
	14	Surface wash	56.1	0.215	73.7	0.514
		Peel [2]	34.7	0.134	21.2	0.148
		Flesh [2]	9.1	0.035	5.1	0.036
		Total	NA	0.384	NA	0.698
	21	Surface wash	51.0	0.302	67.9	0.468
		Peel [2]	38.0	0.224	26.3	0.181
		Flesh [2]	11.0	0.066	5.8	0.040
		Total	NA	0.592	NA	0.689
	30	Surface wash	45.4	0.265	63.2	0.422
		Peel [2]	44.1	0.257	28.6	0.191
		Flesh [2]	10.5	0.061	8.2	0.055
		Total	NA	0.584	NA	0.668
Leaves	0	Surface wash	98.5	53.11	98.0	53.05
		Leaves [2]	1.4	0.75	2.0	1.08
		Total	NA	53.92	NA	54.13
	30	Surface wash	39.9	9.46	48.9	2.25
		Leaves [3]	60.1	14.26	51.1	2.35
		Total	NA	23.72	NA	4.60

[1] Data are the average of 3 or 10 individual fruits and 3 or 6 individual leaves at each interval.
[2] Sum of radioactivity in extracts and residual solids.
[3] Residual solids were <0.2% TRR (<0.003 ppm); estimated to be 0.1% TRR (0.001 ppm) from calculation of sum.
NA = not applicable.

TABLE C.2.1.2 Total Radioactive Residues (TRRs) in/on Apple Leaves and Covered Fruits Following a Single Foliar Application of [^{14}C-PH] or [^{14}C-DOD]-Acequinocyl at 0.637 and 0.691 lb ai/A, Respectively. [1]

Sample	Sampling interval (DAT)	Fraction	[^{14}C-PH]-Acequinocyl		[^{14}C-DOD]-Acequinocyl	
			% TRR	ppm	% TRR	ppm
Fruit	30	Surface wash	NR	0.002	NR	<0.001
		Peel [2]	NR	0.005	NR	0.004
		Flesh [2]	NR	0.010	NR	0.010
		Total	NA	0.016	NA	0.014
Leaves	30	Surface wash	NR	0.048	NR	0.092
		Leaves [2]	NR	0.100	NR	0.078
		Total	NA	0.148	NA	0.166

[1] Data are the average of 4 fruits and 4 leaves.
[2] Sum of radioactivity in extracts and residual solids.
NR = not reported. Percentage distribution was not calculate due to low TRR levels.
NA = not applicable.

TABLE C.2.1.3 Total Radioactive Residues (TRRs) in/on Apple Leaves and Fruits Following a Single Foliar Application of [^{14}C-PH] or [^{14}C-DOD]-Acequinocyl at 0.548 and 0.595 lb ai/A, Respectively. [1]

Sample	Sampling interval (DAT)	Fraction	[^{14}C-PH]-Acequinocyl		[^{14}C-DOD]-Acequinocyl	
			% TRR	ppm	% TRR	ppm
Fruit	28	Surface wash	57.8	0.153	24.8	0.021
		Peel [2]	32.4	0.075	55.4	0.048
		Flesh [2]	9.8	0.022	19.8	0.017
		Total	NA	2.50	NA	0.085
Leaves	28	Surface wash	60.8	4.12	47.3	4.59
		Leaves [2]	39.1	2.62	52.8	5.07
		Total	NA	6.74	NA	9.65

[1] In the supplemental study, data are the average of 2 or 3 batches of fruits (3/batch) and leaves (6/batch).
[2] Sum of radioactivity in extracts and residual solids.
NA = not applicable.

TABLE C.2.2.1. Distribution and Characterization of ^{14}C-Residues in Apple Fruits Following a Single Foliar Application of [^{14}C-PH]Acequinocyl at 0.637 lb ai/A. [1]

Fraction/ Metabolites	Day 0 (TRR = 1.386 ppm)		Day 14 (TRR = 0.384 ppm)		Day 21 (TRR = 0.592 ppm)		Day 30 (TRR = 0.584 ppm)	
	%TRR	ppm	% TRR	ppm	% TRR	ppm	%TRR	ppm
ACN surface rinse (HPLC)	98.7	1.368	56.1	0.215	51.0	0.302	45.4	0.265
Acequinocyl	88.7	1.229	41.2	0.158	30.9	0.183	27.5	0.161
R1	2.6	0.036	2.3	0.009	1.4	0.008	2.2	0.013
AKM-18	0.3	0.004	1.0	0.004	1.4	0.008	2.1	0.012
Phthalic acid [2]	--	--	3.5	0.013	5.9	0.035	3.2	0.019
Minor HPLC/TLC unknowns (each ≤4% TRR)	7.2	0.100	8.2	0.031	11.3	0.067	10.2	0.060
Peel Extract (HPLC) [3]	0.7	0.010	13.2	0.051	14.1	0.083	11.9	0.069
Acequinocyl			6.6	0.025	4.7	0.028	0.9	0.005
R1			<0.1	<0.001	<0.1	<0.001	<0.1	<0.001
AKM-18			<0.1	<0.001	0.2	0.001	<0.1	<0.001
Phthalic acid [2]			3.0	0.012	4.7	0.028	6.1	0.036
Minor HPLC/ TLC unknowns (each <4.4% TRR)			3.6	0.014	4.6	0.028	4.9	0.029
Flesh extract [3]	0.2	0.003	6.1	0.023	6.0	0.036	7.1	0.041
Peel residual solids	0.3	0.004	21.5	0.083	23.9	0.141	32.2	0.188
ACN/water	NA	--	1.5	0.006	1.6	0.009	2.6	0.015
1M NaOH (TLC)	NA	--	17.8	0.068	19.5	0.115	24.7	0.144
Phthalic acid			6.3	0.024	6.5	0.038	6.5	0.038
Minor unknowns (each <5% TRR)			10.1	0.039	10.3	0.060	14.8	0.088
5M NaOH	NA	--	1.5	0.006	2.3	0.014	2.8	0.016
Residual solids	NA	--	0.6	0.002	0.6	0.004	2.1	0.012
Flesh residual solids	<0.1	<0.001	3.0	0.012	5.0	0.030	3.4	0.020

[1] Data are the average of 3 or 10 samples at each interval.
[2] Phthalic acid was identified by TLC analyses of the polar fraction from HPLC analysis.
[3] The peel extract from Day 0 and each of the flesh extracts were not analyzed chromatographically due to low ^{14}C-residue levels.
NA = not applicable.

TABLE C.2.2.2. Distribution and Characterization of ^{14}C-Residues in Apple Fruits Following a Single Foliar Application of [^{14}C-DOD]Acequinocyl at 0.691 lb ai/A. [1]

Fraction/ Metabolites	Day 0 (TRR = 1.302 ppm)		Day 14 (TRR = 0.698 ppm)		Day 21 (TRR = 0.689 ppm)		Day 30 (TRR = 0.668 ppm)	
	%TRR	ppm	% TRR	ppm	% TRR	ppm	%TRR	ppm
ACN surface rinse (HPLC)	98.2	1.279	73.7	0.514	67.9	0.468	63.2	0.422
Acequinocyl	92.0	1.198	57.0	0.398	54.0	0.372	41.1	0.275
R1	1.9	0.025	1.7	0.012	1.8	0.012	4.0	0.027
AKM-18	0.1	0.001	0.8	0.006	0.9	0.006	1.3	0.009
Phthalic acid [2]	--	--	5.4	0.038	3.2	0.022	6.1	0.041
Minor HPLC/ TLC unknowns (each <3.8% TRR)	4.3	0.057	8.8	0.062	8.1	0.057	10.8	0.073
Peel Extract (HPLC) [3]	1.0	0.013	5.9	0.041	7.0	0.048	7.0	0.047
Acequinocyl							0.1	0.001
R1							0.1	0.001
AKM-18							<0.1	<0.001
Phthalic acid [2]							3.2	0.021
Minor HPLC/TLC unknowns (each <2.0% TRR)							3.5	0.024
Flesh extract [3]	0.3	0.004	2.0	0.014	2.6	0.018	3.1	0.021
Peel residual solids	0.3	0.004	15.3	0.107	19.3	0.133	21.6	0.144
ACN/water	NA	--	0.8	0.006	1.1	0.008	1.1	0.007
1M NaOH (TLC)			12.6	0.088	15.0	0.103	16.9	0.113
Phthalic acid			4.0	0.028	5.3	0.037	6.0	0.040
Minor unknowns (each <3.7% TRR)			8.0	0.056	8.4	0.057	9.9	0.065
5M NaOH	NA	--	1.2	0.008	2.0	0.014	2.3	0.015
Residual solids	NA	--	0.6	0.004	1.1	0.008	1.3	0.009
Flesh residual solids	<0.2	<0.003	3.1	0.022	3.2	0.022	5.1	0.034

[1] Data are the average of 3 or 10 samples at each interval.
[2] Phthalic acid was identified by TLC analyses of the polar fraction from HPLC analysis.
[3] The peel extract from Days 0, 14, and 21 and each of the flesh extracts were not analyzed chromatographically due to low ^{14}C-residue levels.
NA = not applicable

TABLE C.2.2.3. Distribution and Characterization of ^{14}C-Residues in Apple Leaves Following a Single Foliar Application of [^{14}C-PH]Acequinocyl at 0.637 lb ai/A. [1]

Fraction/ Metabolites	Day 0 (TRR = 53.92 ppm)		Day 30 (TRR = 23.72 ppm)	
	%TRR	ppm	%TRR	ppm
ACN surface rinse (HPLC)	98.5	53.11	39.9	9.46
Acequinocyl	91.2	49.18	19.4	4.602
R1	1.5	0.809	0.9	0.213
AKM-18	0.3	0.162	2.6	0.617
Phthalic acid [2]	--	--	1.8	0.427
Minor HPLC /TLC unknowns (each <5% TRR)	3.7	2.0	15.3	3.63
Leaf Extract (HPLC) [3]	0.6	0.32	11.8	2.80
Acequinocyl			0.6	0.142
R1			0.3	0.071
AKM-18			0.9	0.213
Phthalic acid [2]			1.8	0.427
Minor HPLC/ TLC unknowns (each <2.8% TRR)			8.5	2.02
Residual solids	0.8	0.43	48.3	11.46
ACN/water (TLC)	NA	--	20.5	4.683
Minor Unknowns (each <6.1% TRR)			19.8	4..815
1M NaOH (TLC)	NA	--	26.3	6.238
Phthalic acid			6.9	1.637
Minor unknowns (each <7.4% TRR)			14.5	3.44
5M NaOH	NA	--	1.0	0.237
Residual solids	NA	--	0.5	0.119

[1] Data are the average of 3 or 6 samples at each interval.
[2] Phthalic acid was identified by TLC analyses of the polar fraction from HPLC analysis.
[3] The extract from Day 0 was not analyzed chromatographically due to low ^{14}C-residue levels.
NA = not applicable.

TABLE C.2.2.4. Distribution and Characterization of ^{14}C-Residues in Apple Leaves Following a Single Foliar Application of [^{14}C-DOD]Acequinocyl at 0.691 lb ai/A. [1]

Fraction/ Metabolites	Day 0 (TRR = 54.13 ppm)		Day 30 (TRR = 4.60 ppm)	
	%TRR	ppm	%TRR	ppm
ACN surface rinse (HPLC)	98.0	53.05	48.9	2.25
Acequinocyl	93.5	50.16	25.6	1.178
R1	1.0	0.541	1.1	0.051
AKM-18	0.2	0.108	3.2	0.147
Phthalic acid [2]	--	--	2.1	0.097
Minor HPLC/TLC unknowns (each <5.3% TRR)	3.4	1.84	16.8	0.774
Leaf Extract (HPLC) [3]	1.5	0.81	13.4	0.62
Acequinocyl			0.4	0.018
R1			0.2	0.009
AKM-18			1.6	0.074
Phthalic acid [2]			1.2	0.055
Minor HPLC/TLC unknowns (each <3.8% TRR)			10.4	0.478
Residual solids	0.5	0.27	37.7	1.73
ACN/water	NA	--	6.1	0.281
1M NaOH (TLC)			28.4	1.306
Phthalic acid			9.1	0.419
Unknown 1X			9.9	0.455
Minor unknowns (each <2.6% TRR)			7.9	0.363
5M NaOH	NA	--	1.1	0.051
Residual solids	NA	--	0.8	0.037

[1] Data are the average of 3 or 6 samples at each interval.
[2] Phthalic acid was identified by TLC analyses of the polar fraction from HPLC analysis.
[3] The extract from Day 0 was not analyzed chromatographically due to low ^{14}C-residue levels.
NA = not applicable.

Table C.2.3.1. Summary of Characterization and Identification of ^{14}C-Residues in/on Apple Fruits Following a Single Foliar Application of [^{14}C-PH]- or [^{14}C-DOD]-Acequinocyl ~0.65 lb ai/A. [1]

[^{14}C-PH]-Acequinocyl								
Metabolites/Fractions [2]	Day 0 (TRR = 1.386 ppm)		Day 14 (TRR = 0.384 ppm)		Day 21 (TRR = 0.592 ppm)		Day 30 (TRR = 0.584 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Acequinocyl	88.7	1.229	47.8	0.183	35.6	0.211	28.4	0.166
Metabolite R1	2.6	0.036	2.3	0.009	1.4	0.008	2.2	0.013
AKM-18	0.3	0.004	1.0	0.004	1.6	0.009	2.1	0.012
Phthalic acid [3]	NA	--	12.8	0.049	17.1	0.101	15.8	0.093
Total identified	**91.6**	**1.269**	**63.9**	**0.245**	**55.7**	**0.329**	**48.5**	**0.284**
Minor Unknowns (each ≤5%TRR) [4]	7.2	0.100	21.9	0.084	26.2	0.155	29.9	0.177
Minor solvent fractions [5]	0.9	0.013	9.1	0.035	9.9	0.059	12.5	0.072
Total characterized	**99.7**	**1.382**	**94.9**	**0.364**	**91.8**	**0.543**	**90.9**	**0.533**
Total extractable [6]	99.7	1.382	96.2	0.369	94.5	0.559	94.5	0.552
Total bound [7]	0.3	0.004	3.6	0.014	5.6	0.034	5.5	0.032
Accountability (% TRR recovered)	100.0		99.8		100.1		100.0	
[^{14}C-DOD]-Acequinocyl								
Metabolites/Fractions [2]	Day 0 (TRR = 1.302 ppm)		Day 14 (TRR = 0.698 ppm)		Day 21 (TRR = 0.689 ppm)		Day 30 (TRR = 0.668 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Acequinocyl	92.0	1.198	57.0	0.398	54.0	0.372	41.2	0.276
Metabolite R1	1.9	0.025	1.7	0.012	1.8	0.012	4.1	0.028
AKM-18	0.1	0.001	0.8	0.006	0.9	0.006	1.3	0.009
Phthalic acid [3]	NA	--	9.4	0.066	8.5	0.059	15.3	0.101
Total identified	**94.0**	**1.224**	**68.9**	**0.482**	**65.2**	**0.449**	**61.9**	**0.414**
Minor Unknowns (each <4%TRR)	4.3	0.057	16.8	0.118	16.5	0.114	24.2	0.162
Minor solvent fractions [5]	1.3	0.017	9.9	0.069	12.7	0.088	6.5	0.043
Total characterized	**99.6**	**1.298**	**95.6**	**0.669**	**94.4**	**0.651**	**92.6**	**0.619**
Total extractable [6]	99.5	1.295	96.2	0.671	95.6	0.659	93.6	0.625
Total bound [7]	0.3	0.004	3.7	0.026	4.3	0.030	6.4	0.043
Accountability (% TRR recovered)	99.8		99.9		99.9		100.0	

[1] For each interval, the data are the average of 3-10 samples, which were analyzed individually. ^{14}C-Residues were quantified by HPLC, except for phthalic acid, which was quantified by TLC.
[2] The chemical names and structures for acequinocyl and its metabolites are presented in Table C.3.1.
[3] Phthalic acid was identified (TLC) as a component of the polar residues from the peel extract.
[4] CBAA was tentatively identified as a minor component of the polar residues from [^{14}C-PH]-treated samples.
[5] Includes any unanalyzed ACN, ACN:water and 5M NaOH extracts.
[6] Total extractable includes ^{14}C-released by solvent and NaOH extractions.
[7] Total bounds radioactivity includes residual solids from peel and flesh.
NA = not applicable.

Table C.2.3.2. Summary of Characterization and Identification of ^{14}C-Residues in/on Apple Leaves Following a Single Foliar Application of [^{14}C-PH]- or [^{14}C-DOD]-Acequinocyl ~0.65 lb ai/A. [1]

[^{14}C-PH]-Acequinocyl				
Metabolites/Fractions [2]	Day 0 (TRR = 53.92 ppm)		Day 30 (TRR = 23.72 ppm)	
	% TRR	ppm	% TRR	ppm
Acequinocyl	91.2	49.18	20.0	4.744
Metabolite R1	1.5	0.809	1.2	0.284
AKM-18	0.3	0.162	3.5	0.830
Phthalic acid	NA	--	10.5	2.491
Total identified	**93.0**	**50.15**	**35.2**	**8.349**
Minor Unknowns (each <7.4%TRR) [4]	3.7	2.0	58.1	13.78
Minor solvent fractions [5]	0.6	0.32	1.0	0.237
Total characterized	**97.3**	**52.47**	**94.3**	**22.366**
Total extractable [6]	99.1	52.47	99.5	23.60
Total bound	0.8	0.43	0.5	0.119
Accountability (% TRR recovered)	99.9		100.0	
[^{14}C-DOD]-Acequinocyl				
Metabolites/Fractions [2]	Day 0 (TRR = 54.13 ppm)		Day 30 (TRR = 4.60 ppm)	
	% TRR	ppm	% TRR	ppm
Acequinocyl	93.5	50.61	26.0	1.196
Metabolite R1	1.0	0.541	1.3	0.060
AKM-18	0.2	0.108	4.8	0.221
Phthalic acid	NA	--	12.4	0.571
Total identified	**94.7**	**51.26**	**44.5**	**2.048**
Polar Unknown 1X	NA	--	9.9	0.455
Minor Unknowns (each <5.3%TRR)	3.4	1.84	35.1	1.615
Minor solvent fractions [5]	1.5	0.81	7.2	0.332
Total characterized	**99.6**	**53.91**	**96.7**	**4.45**
Total extractable [6]	99.5	53.86	97.9	4.503
Total bound	0.5	0.27	0.8	0.037
Accountability (% TRR recovered)	100.0		98.7	

[1] For each interval, the data are the average of 3-6 samples, which were analyzed individually. ^{14}C-Residues were quantified by HPLC, except for polar ^{14}C-residues, which were quantified by TLC.
[2] The chemical names and structures for acequinocyl and its metabolites are presented in Table C.3.1.
[3] Phthalic acid was identified (TLC) as a component of the polar residues from the peel extract.
[4] CBAA was tentatively identified as a minor component of the polar residues from [^{14}C-PH]-treated samples.
[5] Includes any unanalyzed ACN, ACN:water and 5M NaOH extracts.
[6] Total extractable includes radioactivity released by solvent and base extractions.
NA = not applicable.

C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of Acequinocyl in Apple.

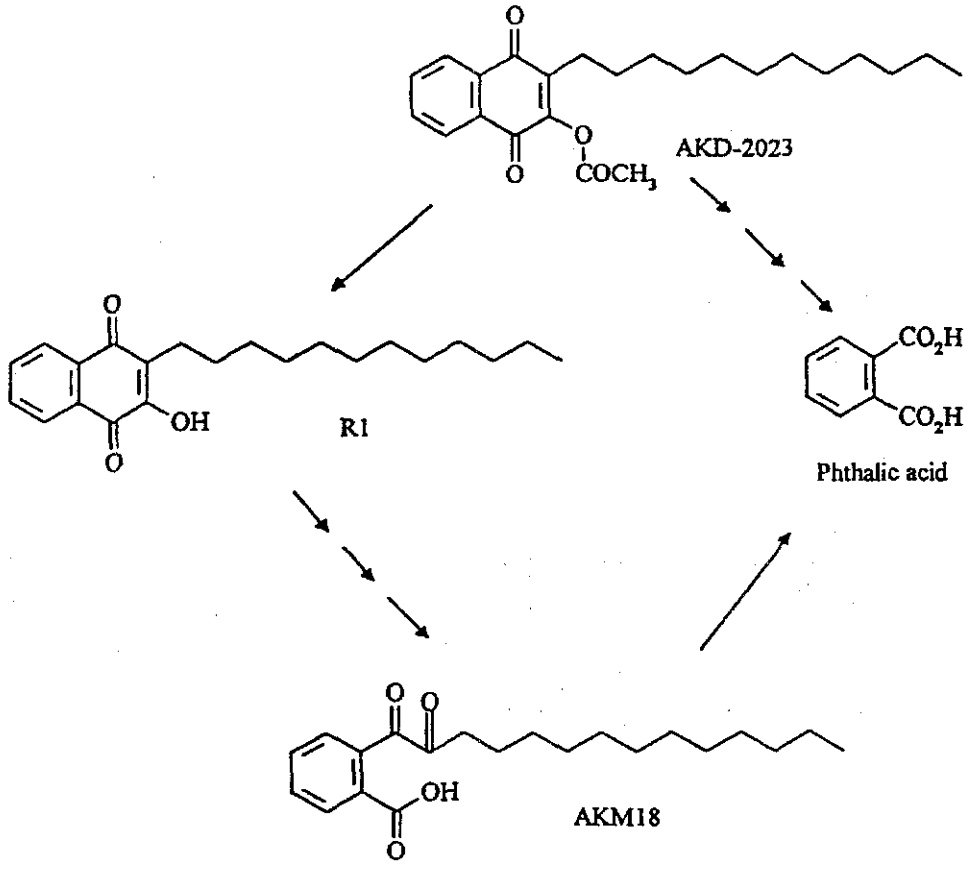
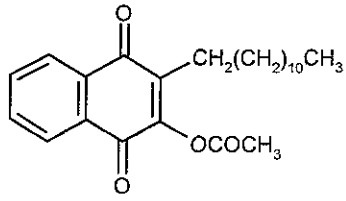
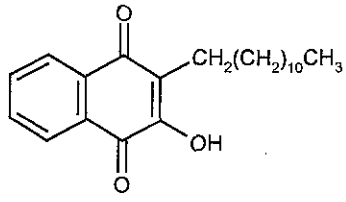
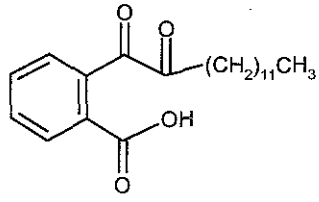
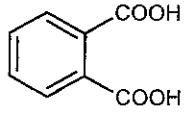
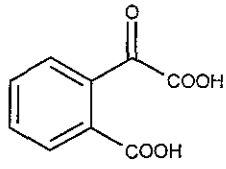


TABLE C.3.1. Identification of Compounds in Apple Metabolism Study.

Common name/code	Chemical name	Chemical structure
Acequinocyl AKD-2023	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione	
Acequinocyl-OH (Metabolite R1)	2-dodecyl-3-hydroxy-1,4-naphthalenedione	
AKM-18	2-(1,2-dioxotetradecyl)-benzoic acid	
phthalic acid		
CBAA [1]	2-carboxy-α-oxo-benzene acetic acid	

[1] Tentatively identified.

D. CONCLUSION

The [^{14}C]-acequinocyl apple metabolism study is adequate, and the metabolite pattern and distribution was similar between the two ^{14}C-labels. Parent was the major ^{14}C-residue identified in/on fruits and leaves at each sampling interval, accounting for 28.4-92.0% of the TRR in/on fruit and 20.0-93.5% of the TRR in/on leaves. Metabolite R1 accounted for 1.4-4.4% of the TRR in fruits and 1.0-1.5% of the TRR in leaves, and Metabolite AKM-18 accounted for 0.1-2.1% of the TRR in fruit and 0.2-4.8% of the TRR in leaves. Phthalic acid were also identified at 8.5-17.1% of the TRR in fruits from 14 to 30 DAT and at 10.5-12.4% of the TRR in leaves from 30 DAT. The remaining residues were comprised of minor unknown components that were primarily polar in nature. Based on the metabolite profile, the metabolism of acequinocyl in apples appears to involve the loss of the acetyloxy moiety to form Metabolite R1, opening of the quinone ring to form AKM-18, and subsequent degradation of the quinone ring to yield phthalic acid.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code 006329/Arvesta
DACO 6.2/OPPTS 860.1300/OECD II 6.2.2, 6.2.3 & IIIA 8.2, 8.4.1, 8.4.2
Nature of the Residues in Plants - Orange

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 26-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651702 McEwen, A. (1999) ^{14}C-AKD 2023 Metabolism in Oranges: Lab Project Number: AGK 40/971376. Unpublished study prepared by Huntingdon Life Sciences Ltd. 117 p.

EXECUTIVE SUMMARY:

In an orange metabolism study, [U-^{14}C-phenyl]-acequinocyl (≥97% radiochemical purity) was formulated as a flowable concentrate (FlC) and applied once as a broadcast foliar application during fruit development to an orange tree growing outdoors. The [^{14}C]acequinocyl was applied at a rate equivalent to 1.05 kg ai/ha (0.94 lb ai/A). Fruit and leaf samples were collected at 0, 14, 21, and 30 days after treatment (DAT).

Average total radioactive residues (TRR) in/on treated fruits declined from 0.633 ppm at 0 DAT to 0.228 ppm by 30 DAT, and average TRR in/on leaves declined from 53.7 ppm at 0 DAT to 25.9 ppm by 30 DAT. Translocation of ^{14}C-residues from leaves to fruits was minimal as indicated by the TRR levels in fruits (0.043 ppm) harvested at 30 DAT from the treated area of tree, but which were covered with plastic bags during the application.

The majority of the TRR in/on fruits and leaves consisted of surface residues, accounting for 97.8% of the TRR immediately following application and declining to 46.9% of the TRR by 30 DAT. ^{14}C-Residue levels in the fruit peel increased over time from 2.2% of the TRR at 0 DAT to 50.5% of the TRR by 30 DAT. ^{14}C-Residues in the flesh were minimal (≤2.95% of the TRR) at all intervals. For leaves, surface ^{14}C-residues accounted for 99.6% of the TRR at 0 DAT and declined to 55.3% of the TRR by 30 DAT.

Surface washes and solvent extractions with acetonitrile (ACN) released 71.5-99.6% of the TRR from fruits and 64.5-99.8% of the TRR from leaves at all sampling intervals. The overall recovery of radioactivity from the fruit and leaf samples was 98.8-103.7%. High-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) analyses identified 44.8-97.5% of the TRR in fruit and 37.7-99.1% of the TRR in leaves, with ^{14}C-residues being identified by co-chromatography with reference standards.

The metabolite profile for fruit and leaves was similar, with acequinocyl being the major ^{14}C-residue in both matrices at each sampling interval. At 0 DAT, acequinocyl accounted for 95.1 and 97.9% of the TRR in/on fruits and leaves, respectively. At the later sampling intervals (14, 21, and 30 DAT), acequinocyl accounted for 35.8-41.4% of the TRR in/on fruits and 27.7-36.4% of the TRR in/on leaves. Metabolites R1 and AKM-18 were identified as minor components, with each accounting for ≤2.1% of the TRR in fruits and ≤6.8% of the TRR in leaves. A substantial portion of the TRR in both fruits and leaves was characterized as being comprised of polar components. One of the polar compounds was identified as phthalic acid, accounting for 7.0-8.2% of the TRR in fruits and 4.9-10.5% of the TRR in leaves. Polar Unknown 1A accounted for 11.3-15.8% of the TRR in fruits and 3.1-7.4% of the TRR in leaves, and polar Unknown 1B accounted for 5.4-7.3% of the TRR in fruits and 7.0-14.5% of the TRR in leaves. Although Unknown 1A was reported to be 2-carboxy-α-oxo-benzene acetic acid (CBBA), no data were provided to support this identification.

Based on the identified metabolites, the metabolism of acequinocyl in oranges appears to involve the loss of the acetyloxy moiety to form Metabolite R1, opening of the quinone ring to form AKM-18, and subsequent degradation of the quinone ring to yield phthalic acid.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The metabolism data on oranges are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the validity of the study.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

TABLE A.1. Nomenclature of Test Compound

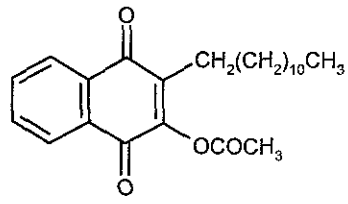
Compound	Chemical Structure
	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	Kanemite™ 15 SC, 1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties

Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 acetone: >250 ethyl acetate: >250 xylene: >250 methanol: 6.1 n-heptane: 36 n-octanol: 29.2	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK$_a$)	no measurable pK$_a$	45434905
Octanol/water partition coefficient Log(K$_{OW}$)	≥6.2	45434906
UV/visible absorption spectrum (nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

The in-life phase of the study was conducted in an established Navel Orange grove by Research for Hire (Porterville, CA) from 07-OCT-1996 to 06-NOV-1996. Separate trees were used for the ^{14}C-label application and the control. For application of the ^{14}C-label, a section (~25 ft^2) of tree containing developing fruits was isolated with plastic sheeting from the remainder of the tree. The treated portion was allowed to dry before the plastic sheeting and bags were removed.

The trials were conducted under ambient environmental conditions, and detailed weather data were provided. During the field study, the maximum daily air temperature ranged from 16 to 39 C and the minimum daily air temperature ranged from 4 to 17 C. Soil temperatures were not reported. Total precipitation was 1.02 inches and was supplemented by 1.17 inches of irrigation. No maintenance fertilizers or pesticides were applied during the trial. Growth and development of the plants was normal, and no problems were noted.

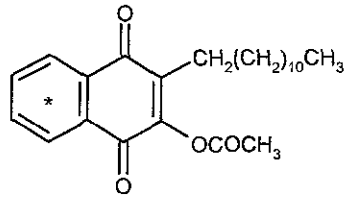
TABLE B.1.1. Test Site Information

Testing Environment	Soil Type
Outdoors in a citrus grove	Clay loam

TABLE B.1.2. Crop Information

Crop	Variety	Growth stage at application	Growth stage at harvest	Harvested Samples
Orange	Navel; *Bonanza* variety	Developing fruit	Normal maturity	Fruit and leaves

B.2. Test Material

TABLE B.2.1. Test Material Characteristics	
Chemical structure	 * indicates ^{14}C-position
Radiolabel position	Uniformly ^{14}C-labeled in the phenyl ring
Batch/Lot No.	CP-1997
Radiochemical Purity [1]	≥98%
Specific activity [1]	1.40 MBq/mg (83,960 dpm/µg)
Code	[^{14}C-PH]

[1] Radiochemical purity (determined by TLC) and specific activity of formulated test substances used for application.

B.3. Study Use Pattern

[^{14}C-PH]-Acequinocyl, formulated as a FlC, was applied to a portion of an orange tree growing in an outdoor grove as a single broadcast foliar application at a rate equivalent to 1.05 kg ai/ha (0.94 lb ai/A, Table B.3.1). The application was made during fruit development, approximately 30 days prior to normal harvest. To examine translocation of ^{14}C-residues, at least 10 fruits in the treated section of the tree were covered with plastic bags during the application.

TABLE B.3.1. Use Pattern Information	
^{14}C-Label	[^{14}C-PH]-Acequinocyl
Application method	Single foliar application to selected branches
Timing of application	During fruit development, ~30 days prior to normal harvest
Application rate	1.05 kg ai/ha (0.94 lb ai/A)
Sampling intervals, PTI (days)	0 (0.5 hours), 14, 21, 30

Duplicate samples of treated fruits and leaves were collected on Day 0 (0.5 hours post-treatment) and at 14, 21, and 30 days after treatment (DAT). Each fruit sample consisted of 5 individual fruits, except for the 30 DAT samples, which consisted of 15 fruits/sample. Each replicate leaf sample consisted for 5 leaves, except for the 30 DAT samples, which consisted of 30 leaves/replicate. At the 30 DAT interval, duplicate samples of fruits (3/sample) protected during application were also collected.

Samples were initially processed (see Section B.4.1) at the field site immediately after sampling, and the resulting samples and sample fractions were stored at -20 C at the field site until overnight shipment on dry ice the analytical laboratory (Huntingdon Life Sciences, LTD., Cambridgeshire, England).

B.4. Identification/Characterization of Residues

B.4.1. Sample Preparation and Extraction

The duplicate samples of fruit and leaves from each interval were analyzed separately. Immediately after collection, fruit and leaf samples were weighed and surface washed three times by sonicating in ACN. The resulting ACN wash, and fruit and leaf samples were then placed in storage at -20 C until shipment to the analytical laboratory.

At the analytical laboratory, fruit were separated into peel and flesh fractions, and ^{14}C-residues in the fruit peel and flesh and leaves were extracted by homogenizing 3 times in ACN, centrifuging after each extraction. Surface rinse and extract fractions containing sufficient radioactivity were analyzed by reverse-phase HPLC and/or normal-phase TLC. Extracts from all fruit flesh samples and peel and leaf extracts from Day 0 were not chromatographically analyzed as ^{14}C-residues in these fractions were low (<2% TRR).

TRR in each sample were determined by summing radioactivity in surface washes, extracts, and residual solids. The data from the analysis of the individual samples were averaged. Radioactivity in liquid fractions was determined by liquid-scintillation counting (LSC) and radioactivity in residual solids was determined by combustion LSC. The limit of detection (LOD) for the radioassays was not reported.

The peel and leaf post-extraction solids (PES) fractions from 14, 21, and 30 DAT were also subjected to further extractions. Residues were shaken and sonicated in ACN:H_2O (1:1, v/v), filtered, and further fractionated by base hydrolysis in 1 M NaOH (2x) at ambient temperature, followed by base hydrolysis in 1 M NaOH at 55 C for 18 hours and then in 5 M NaOH at 55 C for 18 hours. Residues in leaves were then further fractionated by acid hydrolysis in 5 M HCl at ambient temperature, followed by acid hydrolysis in 5 M HCl at 55 C for 18 hours. ^{14}C-Residue released by the ACN:water extraction and the base and acid hydrolyses were not further analyzed.

B.4.2. Analytical Methodology

Radioactive residues in solvent fractions were profiled and quantified by reverse-phase HPLC and/or normal-phase 1D-TLC. For quantifying ^{14}C-residues in solvent fractions, the HPLC systems consisted of a reverse-phase column (C_8 or YMC-AQ) using a mobile phase gradient of 0.015 M ammonium formate (pH 3 or 7) to ACN, with UV and radioactivity detectors. For more detailed analyses of polar components the HPLC systems consisted of a reverse-phase column (Regis SPS) using a mobile phase gradient of ACN to water, with UV and radioactivity detectors.

The TLC analyses used silica gel or reverse-phase plates with a variety (3) of solvent systems. Components were identified by co-chromatography with reference standards.

Including parent, a total of 21 reference standards were used for comparison. Reference standards were detected by (ultraviolet) UV absorbance (254 nm) for HPLC and by UV quenching on TLC plates. Radioactivity eluting from the HPLC was quantified by LSC of collected fractions, and radioactivity on TLC plates was detected and quantified using either a Berthold linear analyzer or a Fuji BAS 200 Bioimage Analyser.

C. RESULTS AND DISCUSSION

The methods used to conduct both the field phase of the orange metabolism study are adequate. Although information on the LOD for the radioassays was not reported, the specific activity of the ^{14}C-test substances and the levels of radioactivity in samples were sufficient to allow for identification and characterization of the ^{14}C-residues.

Following a single foliar application of [^{14}C-PH]-acequinocyl at 0.94 lb ai/A (1.05 kg ai/ha), TRR in/on orange fruits and leaves declined at each sampling interval (Tables C.2.1). Average TRRs in/on fruits were 0.633 ppm at 0 DAT, 0.416 ppm by 14 DAT, 0.238 ppm by 21 DAT, and 0.228 ppm by 30 DAT. Average TRR in/on leaves were 53.7 ppm at 0 DAT, 43.3 ppm by 14 DAT, 39.8 ppm by 21 DAT, and 25.9 ppm by 30 DAT.

Translocation of ^{14}C-residues from treated leaves to adjacent, untreated fruits was minimal. Average TRRs by 30 DAT were 0.043 ppm in/on fruits from the treated area of the tree which were covered during the application.

The majority of the TRR in/on fruit and leaves consisted of surface residues. For the [^{14}C-PH]-treated fruits, surface ^{14}C-residues accounted for the 97.8% of the TRR immediately following application and declined to 46.9% of the TRR by 30 DAT (Table C.2.2.1). ^{14}C-Residue levels in the fruit peel increased over time from 2.2% of the TRR at 0 DAT to 47.4% of the TRR by 14 DAT and 50.5% of the TRR by 30 DAT. ^{14}C-Residues in the flesh were <3% of the TRR at all intervals. For the [^{14}C-PH]-treated leaves, surface ^{14}C-residues accounted for 99.6% of the TRR at 0 DAT and declined to 55.3% of the TRR by 30 DAT.

Surface washes and ACN extractions released 71.5-99.6% of the TRR from fruits and 64.5-99.8% of the TRR from leaves at all sampling intervals. The overall recovery of radioactivity from the fruit and leaf samples was 98.8-103.7%. HPLC and TLC analyses identified 44.7-97.2% of the TRR in fruit and 37.7-99.0% of the TRR in leaves, with ^{14}C-residues being identified by co-chromatography with reference standards.

Acequinocyl was the major ^{14}C-residue in/on orange fruits, accounting for 95.1% of the TRR within 0.5 hours of application and declining to 35.8-41.4% of the TRR at 14, 21, and 30 DAT (Table C.2.3.1). All of the identified metabolites in/on oranges at each sampling interval were

present at ≤8.2% of the TRR, with phthalic acid accounting for <0.1-8.2% of the TRR, AKM-18 accounting for <0.3-0.7% of the TRR, and Metabolite R1 accounting for <0.1-2.1% of the TRR. Two major unknown polar components were also isolated from fruits (TLC Unknowns 1A and 1B). Unknown 1A was a major fraction in orange fruits accounting for 11.3-15.8% of the TRR (0.031-0.047 ppm) from 14 to 30 DAT. The report stated that this unknown was shown to co-chromatograph by HPLC and TLC with CBBA in a supplemental study examining polar metabolites in orange fruit and leaves. However, a copy of this study was not included in the present submission. Unknown 1B accounted for 5.4-7.3% of the TRR in fruits collected from 14 to 30 DAT. Then remaining unknown in fruit each accounted for ≤3.6% of the TRR.

The metabolite profile was similar in leaves. Parent was the major ^{14}C-residue in/on leaves, accounting for 97.9% of the TRR in/on orange leaves at 0.5 hours post-treatment, and declining to 27.7% of the TRR by 30 DAT. As in fruits, all of the identified metabolites in/on leaves were relatively minor components, with phthalic acid accounting for <0.1-10.5% of the TRR, AKM-18 accounting for <0.1-6.8% of the TRR, and R1 accounting for 0.6-3.3% of the TRR. The TLC Unknowns 1A and 1B were also substantial components of the TRR, although in leaves the relative levels of 1B (7.0-14.5% TRR) were higher than 1A (3.1-7.4% TRR) in leaves. The remaining unknowns in leaves released by ACN each accounted for ≤7.7% of the TRR. Large fractions of radioactivity were also released from the leaf PES fractions by ACN:water extraction and mild (1M) base hydrolysis, but these fractions were not analyzed chromatographically. For the 14 to 30 DAT leaf samples, the ACN:water extractions released 6.2-6.4% of the TRR (1.61-2.73 ppm), and the basic extraction at room temperature released 5.3-12.4% of the TRR (2.31-3.20 ppm).

C.1. Storage Stability

Samples of orange leaves and fruits were collected and shipped to the analytical laboratory from 10/14/96 to 12/02/96. The samples were immediately surface washed after collection, but the dates of the sample extractions and analyses were not provided. The experimental termination date was reported to be 11/12/97, therefore the maximum storage interval would theoretically be ~ 1 year. The report stated that all samples and sample fractions were stored at the field site at ca. -20 C. No other storage stability data were provided. Additional sample storage information and stability data are required to support this metabolism study.

TABLE C.1. Summary of Storage Conditions			
Matrix (Extracts)	Storage Temp. (°C)	Actual Study Duration (months) [1]	Limit of Demonstrated Storage Stability (months)
Surface washes, whole fruits and leaves	not provided	≤ 1 year	not available

[1] Study duration is based on the dates of harvest (10/14/96-12/02/96) and on the experimental termination date (11/12/97).

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1 Total Radioactive Residues (TRRs) in/on Orange Fruits and Leaves following a Single Foliar Application of [^{14}C-PH]-Acequinocyl at 0.94 lb ai/A. [1]

Sample	Sampling interval (DAT)	Fraction	[^{14}C-PH]-Acequinocyl	
			% TRR of matrix	ppm
Fruit	0	Surface wash	97.8	0.619
		Peel [2]	2.17	0.015
		Flesh [2]	<0.03	<0.001
		Total	NA	0.633
	14	Surface wash	49.7	0.207
		Peel [2]	47.4	0.197
		Flesh [2]	2.95	0.012
		Total	NA	0.416
	21	Surface wash	43.8	0.104
		Peel [2]	53.7	0.128
		Flesh [2]	2.50	0.006
		Total	NA	0.238
	30	Surface wash	46.9	0.107
		Peel [2]	50.5	0.115
		Flesh [2]	2.7	0.006
		Total	NA	0.228
Untreated Fruit [3]	30	Surface wash	37.2	0.016
		Peel [2]	55.8	0.024
		Flesh [2]	7.0	0.003
		Total	NA	0.043
Leaves	0	Surface wash	99.6	53.5
		Leaves	0.23	0.11
		Total	NA	53.7
	14	Surface wash	69.3	30.0
		Leaves	7.3	3.16
		Total	NA	43.3
	21	Surface wash	65.8	26.2
		Leaves	9.2	3.66

		Total	NA	39.8
	30	Surface wash	55.3	14.3
		Leaves	9.2	2.38
		Total	NA	25.9

1 Data are the average of duplicate samples at each interval.
2 Sum of radioactivity in extracts and residual solids.
3 Fruits in treated section of tree that were protected from direct application by plastic bags.
NA = not applicable.

TABLE C.2.2.1. Distribution and Characterization of ^{14}C-Residues in Orange Fruits Following a Single Application of [^{14}C-PH]-Acequinocyl at ~0.94 lb ai/A. [1]

Fraction/ Metabolites	Day 0 (TRR = 0.633 ppm)		Day 14 (TRR = 0.416 ppm)		Day 21 (TRR = 0.238 ppm)		Day 30 (TRR = 0.228 ppm)	
	%TRR	ppm	% TRR	ppm	% TRR	ppm	%TRR	ppm
ACN surface rinse (HPLC)	97.8	0.619	49.7	0.207	43.8	0.104	46.9	0.107
Acequinocyl	95.1	0.602	35.8	0.149	39.9	0.095	41.4	0.094
AKM-18	<0.3	<0.002	0.7	0.003	<0.3	<0.001	<0.3	<0.001
R1	2.1	0.013	<0.1	<0.001	<0.3	<0.001	0.6	0.001
Minor HPLC unknowns (each ≤3.6% TRR)	0.3	0.002	6.3	0.026	2.0	0.005	1.8	0.004
Polar Fraction (TLC)	0.4	0.003	6.8	0.028	2.0	0.005	3.1	0.007
Phthalic Acid (1C)	NA	--	2.8	0.012	0.5	0.001	1.2	0.003
Polar Unknown 1A			0.6	0.002	0.2	<0.001	0.6	0.001
Polar Unknown 1B			2.5	0.010	0.4	0.001	0.9	0.002
Polar Fraction 1X [2]			0.9	0.004	0.9	0.002	0.4	0.001
Peel Extract (HPLC)	1.82 [3]	0.012	21.0	0.087	26.1	0.062	26.8	0.061
Acequinocyl	NA	--	<0.1	<0.001	<0.1	<0.001	<0.1	<0.001
AKM-18			<0.1	<0.001	0.4	0.001	<0.1	<0.001
R1			<0.1	<0.001	<0.1	<0.001	<0.1	<0.001
Minor HPLC unknowns (each <1.3% TRR)			<0.1	<0.001	0.4	0.001	1.2	0.003
Polar Fraction (TLC)			21.0	0.087	25.4	0.060	25.6	0.058
Phthalic Acid (1C)			5.4	0.022	6.7	0.016	5.8	0.013
Polar Unknown 1A			10.7	0.045	12.8	0.030	15.2	0.035
Polar Unknown 1B			4.8	0.020	5.0	0.012	4.6	0.010
Polar Fraction 1X [2]			0.2	0.001	0.9	0.002	ND	--
Flesh extract [3]	<0.03	<0.001	2.14	0.009	1.64	0.004	ND	--
Flesh residual solids	<0.03	<0.001	0.81	0.003	0.86	0.002	2.7	0.006
Peel residual solids	0.35	0.003	26.4	0.110	27.6	0.066	23.7	0.054
ACN:H_2O extract	NA	--	5.87	0.024	5.36	0.013	6.26	0.013
1 M NaOH			4.48	0.019	5.22	0.012	3.94	0.008
1 M NaOH (18 hr. at 55 C)			7.89	0.033	6.30	0.015	4.54	0.009

5 M NaOH (18 hr. at 55 C)			4.20	0.017	4.12	0.010	2.72	0.006
Residual Solids			4.00	0.017	6.70	0.016	6.20	0.013

[1] Data are the average of 2 samples at each interval.
[2] TLC fraction 1X was radioactivity that essentially remained at the origin.
[3] The peel extracts from Day 0 and each of the flesh extracts were not analyzed chromatographically due to low ^{14}C-residue levels.

NA = not applicable.
ND = not detected.

TABLE C.2.2.2. Distribution and Characterization of ^{14}C-Residues in Orange Leaves Following a Single Application of [^{14}C-PH]-Acequinocyl at ~0.94 lb ai/A. [1]

Fraction/ Metabolites	Day 0 (TRR = 53.67 ppm)		Day 14 (TRR = 43.29 ppm)		Day 21 (TRR = 39.79 ppm)		Day 30 (TRR = 25.85 ppm)	
	%TRR	ppm	% TRR	ppm	% TRR	ppm	%TRR	ppm
ACN surface rinse (HPLC)	99.6	53.5	69.3	30.0	65.8	26.2	55.3	14.3
Acequinocyl	97.9	52.54	29.5	12.77	36.2	14.40	27.7	7.16
AKM-18	<0.1	<0.054	6.2	2.68	<0.9	<0.358	1.1	0.284
R1	1.1	0.590	<0.5	<0.216	<0.9	<0.358	3.3	0.851
Minor HPLC unknowns (each ≤7.7% TRR)	0.6	0.322	13.5	5.84	1.1	0.438	11.4	2.95
Polar Fraction (TLC)	<0.1 [2]	<0.054	20.0	8.66	28.7	11.42	11.7	3.02
Phthalic Acid (1C)	NA	--	5.7	2.48	8.2	3.26	2.7	0.698
Polar Unknown 1A			3.3	1.43	5.6	2.22	1.3	0.336
Polar Unknown 1B			8.6	3.71	11.3	4.50	3.9	1.01
Polar Fraction 1X [3]			2.5	1.08	3.7	1.47	3.7	0.956
Leaf Extract (HPLC)	0.23 [2]	0.11	7.3	3.16	9.2	3.66	9.2	2.38
Acequinocyl	NA	--	0.4	0.173	0.2	0.080	<0.1	<0.026
AKM-18			0.6	0.260	0.3	0.119	0.7	0.181
R1			<0.1	<0.043	0.2	0.080	<0.1	<0.026
Minor HPLC unknowns (each ≤1.4% TRR)			1.2	0.216	0.98	0.390	1.4	0.362
Polar Fraction (TLC)			5.3	2.29	7.3	2.91	7.1	1.84
Phthalic Acid (1C)			1.8	0.779	2.3	0.915	2.2	0.569
Polar Unknown 1A			0.8	0.346	1.8	0.716	1.8	0.465
Polar Unknown 1B			2.7	1.17	3.2	1.27	3.1	0.801
Polar Fraction 1X [3]			ND	--	ND	--	ND	--
Residual solids	0.20 [2]	0.11	23.4	10.13	25.0	9.95	35.6	9.20
ACN:H_2O extract	NA	--	6.30	2.73	6.44	2.56	6.24	1.61
1 M NaOH			5.34	2.31	7.05	2.81	12.4	3.20
1 M NaOH (18 hr. at 55 C)			3.86	1.67	3.86	1.54	3.74	0.97
5 M NaOH (18 hr. at 55 C)			1.10 [4]	0.48	0.70	0.28	3.26	0.84
5 M HCl			0.44 [4]	0.19	0.43	0.17	0.65	0.17
1 M HCl (18 hr. at 55 C)			0.30 [4]	0.13	0.29	0.12	0.30	0.08
Residual Solids			6.10	2.62	6.20	2.48	9.00	2.33

[1] Data are the average of 2 samples at each interval, unless otherwise noted.
[2] The polar fraction from the leaf wash, leaf extracts, and the residual solids from Day 0 were not analyzed chromatographically due to low ^{14}C-residue levels.
[3] TLC fraction 1X was radioactivity that essentially remained at the origin.
[4] Data are from one sample.
NA = not applicable.
ND = not detected.

TABLE C.2.3.1. Summary of Characterization and Identification of ^{14}C-Residues in/on Orange Fruit Following a Single Foliar Application of [^{14}C-PH]-Acequinocyl at 0.94 lb ai/A. [1]

Metabolites/Fractions [2]	Day 0 (TRR = 0.633 ppm)		Day 14 (TRR = 0.416 ppm)		Day 21 (TRR = 0.238 ppm)		Day 30 (TRR = 0.228 ppm)	
	%TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Acequinocyl	95.1	0.602	35.8	0.149	39.9	0.095	41.4	0.094
AKM-18	<0.3	<0.002	0.7	0.003	0.4	0.001	<0.4	<0.001
Metabolite R1	2.1	0.013	<0.1	<0.001	<0.3	<0.001	0.6	0.001
Phthalic Acid [3]	NA	--	8.2	0.033	7.2	0.017	7.0	0.016
Total identified	**97.2**	**0.615**	**44.7**	**0.187**	**47.5**	**0.113**	**49.0**	**0.111**
Polar Unknown 1A [4]	NA	--	11.3	0.047	13.0	0.031	15.8	0.036
Polar Unknown 1B	NA	--	7.3	0.030	5.4	0.013	5.5	0.012
Minor Unknowns (each ≤3.6%TRR)	0.7	0.004	7.4	0.031	5.6	0.014	3.4	0.008
Minor solvent fractions [5]	1.8	0.011	24.6	0.102	22.6	0.054	15.6	0.036
Total characterized	**98.7**	**0.630**	**95.3**	**0.397**	**94.1**	**0.225**	**89.7**	**0.203**
Total extractable [6]	99.4	0.630	95.8	0.397	92.6	0.223	89.9	0.207
Total bound [7]	0.35	0.003	4.8	0.020	7.5	0.018	8.9	0.019
Accountability (% TRR recovered)	99.8		100.6		100.1		98.8	

[1] For each interval, the data are the average of 2 samples, which were analyzed individually. ^{14}C-Residues were quantified by HPLC, except for phthalic acid, which was quantified by TLC.
[2] The chemical names and structures for acequinocyl and its metabolites are presented in Table C.3.1.
[3] Phthalic acid was identified (TLC) as a component of the polar residues.
[4] The report stated that Polar Unknown 1A was identified by HPLC and TLC as CBBA in a separate report; however, this report was not provided for review.
[5] Includes fractions released by further extraction of peel PES; each fraction accounted for ≤7.9% TRR
[6] Total extractable includes ^{14}C-released by solvent extraction and by acid and base hydrolyses.
[7] Total bound radioactivity includes residual solids from peel and flesh.
ND = not detected

TABLE C.2.3.2. Summary of Characterization and Identification of ^{14}C-Residues in/on Orange Leaves Following a Single Foliar Application of [^{14}C-PH]-Acequinocyl at 0.94 lb ai/A. [1]

Metabolites/Fractions [2]	Day 0 (TRR = 53.67 ppm)		Day 14 (TRR = 43.29 ppm)		Day 21 (TRR = 39.79 ppm)		Day 30 (TRR = 25.85 ppm)	
	%TRR	ppm	% TRR	ppm	% TRR	ppm	% TRR	ppm
Acequinocyl	97.9	52.5	29.9	12.94	36.4	14.48	27.7	7.16
AKM-18	<0.1	<0.05	6.8	2.94	0.3	0.12	1.8	0.47
Metabolite R1	1.1	0.59	<0.5	<0.22	0.2	0.08	3.3	0.85
Phthalic Acid [3]	NA	--	7.5	3.26	10.5	4.18	4.9	1.27
Total identified	**99.0**	**53.09**	**44.2**	**19.14**	**47.4**	**18.86**	**37.7**	**9.75**
Polar Unknown 1A [4]	NA	--	4.1	1.78	7.4	2.94	3.1	0.80
Polar Unknown 1B	NA	--	11.3	4.88	14.5	5.77	7.0	1.81
Minor Unknowns (each ≤7.7%TRR)	0.6	0.322	16.5	7.14	5.78	2.30	16.5	4.27
Minor solvent fractions [5]	0.23	0.11	17.3	7.51	18.8	7.48	26.6	6.87
Total characterized	**99.8**	**53.52**	**93.4**	**40.45**	**93.9**	**37.35**	**90.9**	**23.50**
Total extractable [6]	100.1	53.72	94.8	41.04	97.5	38.80	91.1	23.60
Total bound	0.20	0.11	6.1	2.60	6.2	2.5	9.0	2.3
Accountability (% TRR recovered)	100.3		100.9		103.7		100.1	

[1] For each interval, the data are the average of 2 samples, which were analyzed individually. ^{14}C-Residues were quantified by HPLC, except for Phthalic acid, which was quantified by TLC.

[2] The chemical names and structures for acequinocyl and its metabolites are presented in Table C.3.1.

[3] Phthalic acid was identified (TLC) as a component of the polar residues.

[4] The report stated that Polar Unknown 1A was identified by HPLC and TLC as CBBA in a separate report; however, this report was not provided for review.

[5] Includes ^{14}C-residues released by ACN:water extraction and acid and base hydrolyses of the leaf PES fraction. Each fraction accounted for ≤7.1% TRR, with the exception of the 1M NaOH extract from the 30 DAT sample (12.4% TRR, 3.20 ppm)

[6] Total extractable includes ^{14}C-released by solvent extraction and by acid and base hydrolyses.

ND = not detected
NA = not applicable.

C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of [^{14}C]Acequinocyl in Oranges.

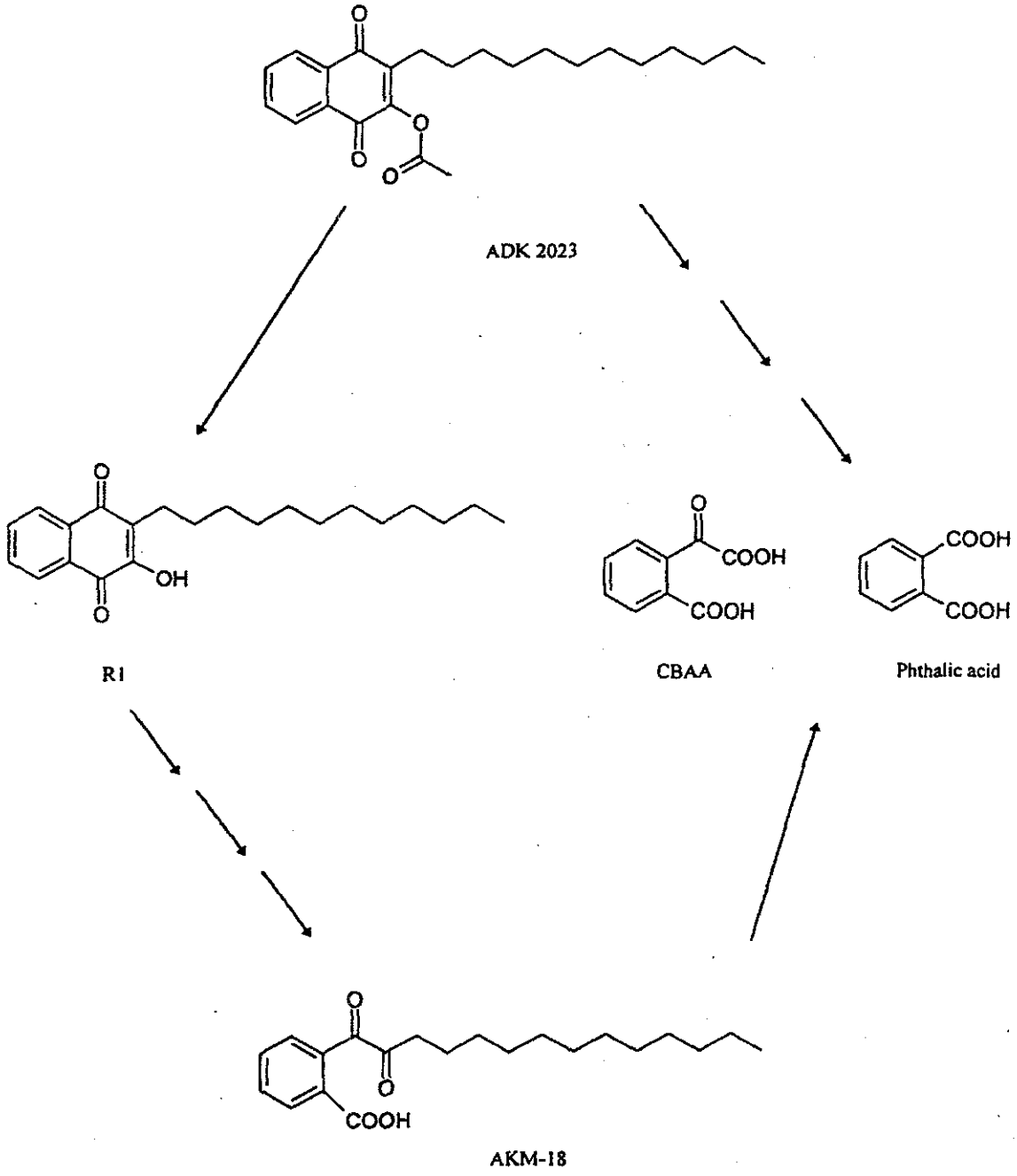
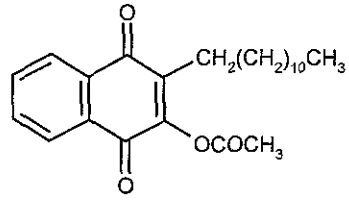
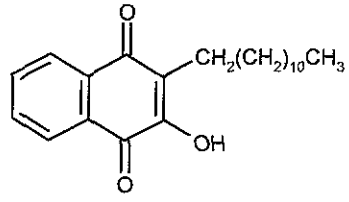
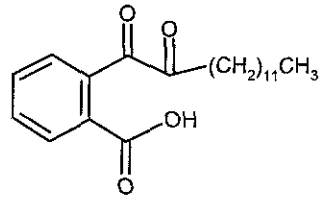
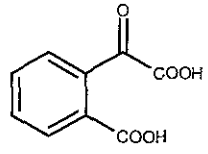
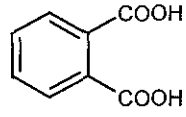


TABLE C.3.1. Identification of Compounds in Orange Metabolism Study.

Common name/code	Chemical name	Chemical structure
Acequinocyl AKD-2023	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione	
Acequinocyl-OH (Metabolite R1)	2-dodecyl-3-hydroxy-1,4-naphthalenedione	
AKM-18	2-(1,2-dioxotetradecyl)-benzoic acid	
CBAA [1]	2-carboxy-α-oxo-benzene acetic acid	
phthalic acid		

[1] CBAA was apparently identified as a component of the polar ^{14}C-residues (Unknown 1A) in a separate report that was not provided.

D. CONCLUSION

The [^{14}C]-acequinocyl orange metabolism study is adequate. Based on the identified metabolites, the metabolism of acequinocyl in oranges appears to involve the loss of the acetyloxy moiety to form Metabolite R1, opening of the quinone ring to form AKM-18, and subsequent degradation of the quinone ring to yield phthalic acid.

F. REFERENCES

None

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code 006329/Arvesta
DACO 6.3/OPPTS 860.1300/OECD II 6.2.2, 6.2.3 & IIIA 8.2, 8.4.1, 8.4.2
Nature of the Residues in Plants - Eggplant

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 03-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651703 Mayo, B. (1997) ^{14}C-AKD 2023 Metabolism in Egg Plants: Lab Project Number: AGK 20/950490. Unpublished study prepared by Huntingdon Life Sciences, Ltd. and Arvesta Corporation. 233 pages.

EXECUTIVE SUMMARY:

In an eggplant metabolism study, [U-^{14}C-phenyl]- or [1-^{14}C-dodecyl]acequinocyl (≥95% radiochemical purity) was formulated as a flowable concentrate (FlC) and applied once as a broadcast foliar or soil application during fruit development to eggplants grown in growth chambers. The [^{14}C]acequinocyl was applied at rates equivalent to 0.55-0.62 kg ai/ha (0.49-0.55 lb ai/A). Fruit and leaf samples were collected at 0, 7, and 14 days after treatment (DAT).

The levels of total radioactive residues (TRR) in/on eggplant fruits and leaves were quite variable, but were generally similar for the two ^{14}C-labels. For fruits, average TRR were 0.116-0.374 ppm at 0 DAT, 0.087-0.156 ppm at 7 DAT, and 0.061-0.140 ppm at 14 DAT. For leaves, average TRR were 13.98-26.60 ppm at 0 DAT, 13.04-22.60 ppm at 7 DAT, and 5.00-7.21 ppm at 14 DAT. Translocation of ^{14}C-residues from leaves to fruits and uptake from the soil was minimal. When fruits were covered prior to foliar application of either ^{14}C-label, TRR in/on fruits were 0.005-0.031 ppm at 14 DAT. When either ^{14}C-label was applied to soil, TRR were 0.005-0.012 ppm in/on fruit and 0.018-0.031 ppm in/on leaves at 14 DAT.

Following a foliar application, the majority of the TRR consisted of surface residues. Surface ^{14}C-residues in/on fruit and leaves accounted for the 95.1-98.2% of the TRR at 0 DAT and 60.7-79.8% of the TRR at 14 DAT. The percentage of the fruit TRR associated with the fruit peel and flesh increased over time. By 14 DAT, radioactivity in the peel accounted for 18.0-28.6% of the TRR and radioactivity in the flesh accounted for 6.6-11.4% of the TRR.

Surface washes and solvent extractions released 83.6-99.5% of the TRR from fruits and 73.2-99.7% of the TRR from leaves at all sampling intervals. Enzymatic digestion and mild base extraction released an additional 4.8-19.7% of the TRR. The overall recovery of radioactivity from the fruit and leaf samples was 97.1-104.9%. High-performance liquid chromatography

(HPLC) and thin-layer chromatography (TLC) analyses identified 53.3-89.8% of the TRR in fruit and 47.5-94.5% of the TRR in leaves, with ^{14}C-residues being identified by co-chromatography with reference standards. Sufficient information was available to assess the stability of ^{14}C-residues; no additional sample storage information or stability data are required.

The metabolite profile in/on fruits and leaves was similar for both the [^{14}C-phenyl]- and [^{14}C-dodecyl]-labels. Acequinocyl was the major ^{14}C-residue in/on fruits and leaves at all sampling intervals. In fruit, acequinocyl accounted for 81.6-83.7% of the TRR at 0 DAT and 46.2-58.4% of the TRR at 7 and 14 DAT; and in leaves, acequinocyl accounted for 89.0-92.1% of the TRR at 0 DAT, 51.8-74.9% of the TRR at 7 DAT, and 38.4-58.1% of the TRR by 14 DAT. Metabolite R1 was a minor component of the residue in leaves and fruits at each interval, accounting for 4.3-10.3% of the TRR in fruits and 1.0-4.1% of the TRR in leaves. Minor amounts of AKM-18 (0.3-6.7% TRR) were also identified in fruits and leaves at each interval. The remaining solubilized ^{14}C-residues in leaves and fruits were comprised primarily of minor polar metabolites, each accounting for <10% of the TRR. Although quantitative data were limited, one of the unknown fractions (1C) was identified as phthalic acid, and two other polar unknowns were characterized as minor acidic metabolites that degraded to phthalic acid following base hydrolysis.

Based on the identified metabolites, the metabolism of acequinocyl in eggplants appears to involve the loss of the acetyloxy moiety to form Metabolite R1, opening of the quinone ring to form AKM-18, and subsequent degradation of the quinone ring to yield phthalic acid.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the eggplant metabolism data are classified as scientifically acceptable. Although the exact dates of the sample extractions and analyses were not provided, sufficient information was available to assess the stability of ^{14}C-residues.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance and Data Confidentiality statements were provided.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

TABLE A.1. Test Compound Nomenclature

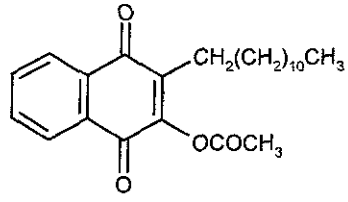
Compound	Chemical Structure
	
Common name	Acequinocyl
Company experimental name	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use product/EP	Kanemite™ 15 SC, 1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties

Parameter	Value	Reference (MRID#)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20˚C)	6.69 µg/L	45434906
Solvent solubility (mg/L at 20˚C)	1,2-dichloroethane: >250 acetone: >250 ethyl acetate: >250 xylene: >250 methanol: 6.1 n-heptane: 36 n-octanol: 29.2	45434904
Vapor pressure at 25˚C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Test Site and Crop Information

The in-life and analytical phases of the study were conducted by Huntingdon Life Sciences Ltd. (Cambridgeshire, England) from 29-JUL-1994 to 02-JUL-1996. Eggplants were grown individually in plastic pots containing a peat based compost in an indoor growth room (17-28 C) with lighting provided by metal halide lamps (15-hour daylength). Plants were watered regularly and supplied periodically with liquid fertilizer. Growth and development of the plants was normal, and no problems were noted.

TABLE B.1.1. Test Site Information

Testing Environment	Soil characteristics			
	Type	%OM	pH	CEC
Plants were grown in individual plastic pots (area ≈ 0.1 m^2) in growth rooms with artificial lighting	Peat-based potting soil	NR	NR	NR

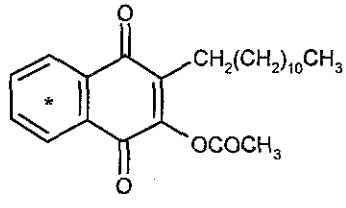
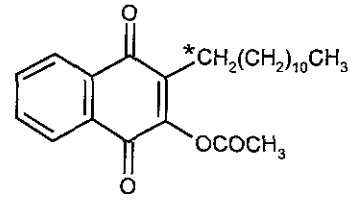
NR = not reported.

TABLE B.1.2. Crop Information

Crop/crop group	Variety	Growth stage at application	Growth stage at harvest	Harvested RAC	Harvesting procedure
Eggplant (*Solanum Melongena*)/ Fruiting Vegetable	Senryo	Fruit development	Mature	Fruit and leaves	Hand

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics

Chemical structure	 * indicates ^{14}C-position	 * indicates ^{14}C-position
Radiolabel position	Uniformly ^{14}C-labeled in the phenyl ring	^{14}C-labeled at the C-1 position of the dodecyl side chain
Lot No.	CP-1526	CP-1525
Radiochemical Purity [1]	≥95%	≥95%
Specific activity [1]	2.28 MBq/mg (137,000 dpm/μg)	2.15 MBq/mg (129,000)
Code	[^{14}C-PH]	[^{14}C-DOD]

[1] The test substances were formulated as a FlC and diluted with water for application. The radiochemical purity and specific activity of formulated test substances.

B.3. Study Use Pattern

[^{14}C-PH]- and [^{14}C-DOD]-Acequinocyl, formulated as a FlC, was applied separately to eggplants growing in pots, as a single broadcast foliar application at a rates equivalent to 573 and 621 g ai/ha, respectively (Table B.3.1). The application was made during fruit development, approximately 14 days prior to normal harvest. To examine translocation, the fruits on selected plants were covered with plastic bags during application. In addition, selected plants received a single soil application of either [^{14}C-PH]- and [^{14}C-DOD]-acequinocyl (FlC) during fruit development at rates equivalent to 486 and 526 g ai/ha, respectively. To provide samples for examination of polar metabolites, an additional study was conducted in which eggplants received a single broadcast foliar application of [^{14}C-PH]-acequinocyl at rates of 498 or 3,363 g ai/ha during fruit development. In the main study, [^{14}C-PH]-acequinocyl was applied on 7/29/94 and the [^{14}C-DOD]-acequinocyl was applied on 8/4/94. In the supplemental study, the [^{14}C-PH]-acequinocyl was applied on 7/2/96.

TABLE B.3.1. Use Pattern Information

Main Study				
Chemical name	[^{14}C-PH]Acequinocyl		[^{14}C-DOD]Acequinocyl	
Application method	foliar	soil	foliar	soil
Application rate (kg ai/ha)	0.573 (0.510 lb ai/A)	0.546 (0.486 lb ai/A)	0.621 (0.553 lb ai/A)	0.591 (0.526 lb ai/A)
Number of applications	1	1	1	1
Timing of applications	Broadcast foliar application during flowering and fruit development			
PHI(s) for leaves and fruits	0, 7, 14	14	0, 7, 14	14
Supplemental Study				
Chemical	[^{14}C-PH]Acequinocyl			
Application method	Broadcast foliar application during flowering and fruit development			
Application rate (kg ai/ha)	0.498 (0.443 lb ai/A)		3.363 (2.99 lb ai/A)	
Number of applications	1		1	
Timing of applications	Broadcast foliar application during fruit development			
PHI(s) for leaves	14, 28		14, 28	

Following the broadcast foliar application with each ^{14}C-label, 2-5 individual fruits and leaves were collected at 0 (<0.5 hour), 7, and 14 DAT. Two or four samples of fruit, which were covered during application, were also collected at 7 and 14 DAT. Following soil application of each ^{14}C-label, a single soil sample was collected at 0 and 14 DAT, and four fruit samples and ten leaf samples were collected at maturity (14 DAT). In the supplemental study, one-third of the leaves from each plant were collected at 14 DAT and the remaining two-thirds of the leaves were collected at 28 DAT. All samples were stored at <-15 C.

B.4. Identification/ Characterization of Residues

B.4.1. Sample Preparation

Immediately following collection, individual fruit and leaf samples were surface rinsed three times by sonicating in acetonitrile (ACN). Fruit samples were then divided into peel and flesh fractions. ^{14}C-Residues in the individual peel, flesh, and leaf samples were then extracted by homogenizing 3 times in ACN, centrifuging after each extraction. Extract fractions were pooled by sample and concentrated at 37 C. Surface rinse and extract fractions containing sufficient radioactivity were analyzed by reverse-phase HPLC and normal-phase TLC.

In the main study, TRR in each sample were determined by summing radioactivity in surface washes, extracts, and residual solids. The data from the analysis of the individual samples were reported as averages with standard deviation. In the supplemental study, the leaves were pooled into one sample per interval for analysis. Radioactivity in liquid fractions was determined by liquid scintillation counting (LSC) and radioactivity in residual solids was determined by combustion LSC. The limit of detection (LOD) for the radioassays was not reported.

The post-extraction solid (PES) fractions from peel samples from 7 DAT (^{14}C-DOD-label) and 14 DAT (both ^{14}C-labels) and the PES fractions from leaf samples from 7 DAT (^{14}C-PH-label) and 14 DAT (both ^{14}C-labels) were subjected to further extractions. Subsamples of pooled PES fractions were incubated with a mixture of cellulase, hemicellulase, pectinase, and β-glucosidase in 0.1M sodium acetate buffer at pH 5 for 18-24 hours at 37 C. The resulting enzyme digests were then extracted repeatedly with ACN, centrifuging after each extraction. The resulting extracts were analyzed by TLC.

Solids remaining after digestion of the [^{14}C-PH]-treated leaf samples (7 and 14 DAT) were further extracted with 1M NaOH at room temperature for 2 hours. Solubilized ^{14}C-residues were analyzed by TLC.

To further examine the nature of the polar ^{14}C-residues in eggplants, leaves (14 and 28 DAT) from plants treated with [^{14}C-PH]-acequinocyl at 0.5 or 3.63 kg ai/ha (0.44 or 2.99 lb ai/A) in the supplemental study were surface rinsed and extracted as described above for the main study. The resulting surface rinse and leaf extract from the 14 DAT samples was analyzed by HPLC as in the main study. In addition, the surface rinse was partitioned with hexane to remove the more non-polar ^{14}C-residues. The polar ^{14}C-residues were concentrated to dryness, redissolved in water, and purified using an XAD-4 ion exchange column eluted sequentially with water, methanol, and methanol containing 1% trifluoroacetic acid. The metabolites were further purified by TLC. The partially purified polar metabolites were then subjected to a series of tests. The partitioning behavior of the polar metabolites into ethyl acetate was examined at pH 3, 7, and 11. The metabolites were acid (2M HCl) or base (2M NaOH) hydrolyzed at 37 C for ~20 hours or at 100 C for 1 hours, and the resulting hydrolysates were neutralized and analyzed by TLC or HPLC. Polar metabolites were also subjected to enzymatic hydrolyses with either pectinase, a mixture of cellulase, hemicellulase, β-glucosidase, and xylanase, or protease. The

hydrolysates were then examined by TLC. Finally selected fractions were derivatized with diazomethane and analyzed by TLC and HPLC.

B.4.2. Analytical Methodology

Radioactive residues in solvent fractions were profiled and quantified by reverse-phase HPLC and/or normal-phase 1D- and 2D-TLC. For quantifying ^{14}C-residues in solvent fractions, the HPLC systems consisted of a reverse-phase column (C_8 or PRP-1) using a mobile phase gradient of 0.015 M ammonium formate (pH 7) to ACN, with ultraviolet (UV) and radioactivity detectors. For more detailed analyses of polar components, the HPLC systems consisted of a reverse-phase column (YMC-AQ or Hypersil H10) using a mobile phase gradient of 0.1 or 0.01 M ammonium formate (pH 3 or 4) to ACN, with UV and radioactivity detectors. The TLC analyses used silica gel or reverse-phase plates with a variety (6) of solvent systems. Components were identified by co-chromatography with reference standards on at least two systems.

Including parent, a total of 22 reference standards were used for comparison. Reference standards were detected by UV absorbance (254 nm) for HPLC and by UV quenching on TLC plates. Radioactivity eluting from the HPLC was quantified by LSC of collected fractions, and radioactivity on TLC plates was detected and quantified using either a Berthold linear analyzer (1D-TLC) or a Fuji BAS 2000 Bioimage Analyzer (2D-TLC).

C. RESULTS AND DISCUSSION

The methods used to conduct both the in-life and analytical phases of the eggplant metabolism study are adequate. Although information on the LOD for the radioassays was not reported, the specific activity of the ^{14}C-test substances and the levels of radioactivity in the harvested samples were sufficient to allow for identification and characterization of the ^{14}C-residues.

Following a single foliar application of either [^{14}C-PH] or [^{14}C-DOD]-acequinocyl (FlC) at 0.57 and 0.62 kg ai/ha (0.51 and 0.55 lb ai/A), TRR in/on eggplant fruits and leaves were quite variable, but were generally similar for the two ^{14}C-labels (Table C.2.1.1). Coefficients of variation (CV) for the average TRR ranged from 22-115% for fruits and 31-70% for leaf samples. On the day of application, average TRRs were 0.116 and 0.374 ppm in/on fruits and 13.98 and 26.60 ppm in/on leaves from the [^{14}C-PH] and [^{14}C-DOD]-labels, respectively. Decline in TRR values was also variable. TRR in/on [^{14}C-PH]-treated fruits initially declined to 0.087 ppm at 7 DAT and then increased to 0.140 ppm by 14 DAT; whereas, TRR in/on [^{14}C-DOD]-treated fruits declined steadily to 0.061 ppm by 14 DAT. For leaves, TRR declined to 7.21 ppm in/on [^{14}C-PH]-treated leaves and 5.00 ppm in/on [^{14}C-DOD]-treated leaves by 14 DAT.

For fruit and leaf samples from both ^{14}C-labels, the majority of the TRR consisted of surface residues. Immediately following application (0 DAT), surface ^{14}C-residues on eggplant fruits accounted for the 95.1-97.9% of the TRR, and by 14 DAT, surface ^{14}C-residues on fruit accounted for the 60.7-78.7% of the TRR. Similarly, surface ^{14}C-residues on leaves accounted for the 97.9-98.2% of the TRR at 0 DAT and 67.8-79.8% of the TRR by 14 DAT. The percentage of the fruit TRR associated with the peel and flesh increased over time. By 14 DAT, radioactivity in the peel accounted for 18.0-28.6% of the TRR and radioactivity in the flesh accounted for 6.6-11.4% of the TRR.

Translocation of ^{14}C-residues from leaves to fruits was minimal. When fruits were covered prior to foliar application of either ^{14}C-label, TRR in/on fruits were ≤0.004 ppm at 7 DAT and 0.005-0.031 ppm at 14 DAT (Table C.2.1.2). On the same plants, TRR in/on leaves were 13.44-49.32 ppm at 7 DAT and 6.00-11.15 ppm at 14 DAT.

Uptake of [^{14}C]acequinocyl from the soil was also limited. Following a single soil application of [^{14}C-PH]- or [^{14}C-DOD]-acequinocyl at 0.49 and 0.53 lb ai/A, respectively, TRR were 0.005-0.012 ppm in/on fruit and 0.018-0.031 ppm in/on leaves at 14 DAT (Table C.2.1.3).

The distribution and fractionation of radioactivity in/on fruits and leaves following foliar application of the two ^{14}C-labels were similar (Tables C.2.2.1-C.2.2.4). Surface washes with ACN and solvent extractions released 83.6-99.5% of the TRR from fruits at all sampling intervals, with the peel extracts accounting for 11.5-35.9% of the TRR at 7 and 14 DAT. At each sampling interval, flesh extracts accounted for ≤3.6% of the TRR (≤0.005 ppm). Post-extraction solids (PES) from peel and flesh accounted for 10.1-10.3% of the TRR (≤0.016 ppm) at 7 DAT and 9.9-17.2% of the TRR (≤0.024 ppm) at 14 DAT. Enzymatic digestion of the peel PES fractions from 14 DAT released an additional 4.8-6.1% of the TRR. The overall recovery of radioactivity from the fruit samples was 98.7-104.9%.

For leaves, surface washes and solvent extractions released ≥99.7% of the TRR at 0 DAT, 78.7-95.6% of the TRR at 7 DAT and 73.2-85.8% of the TRR at 14 DAT, with extractability of [^{14}C-DOD]-treated samples being somewhat higher than for the [^{14}C-PH]-treated samples. By 14 DAT, surface residues accounted 67.8-79.8% of the leaf TRR, leaf extracts accounted for 5.4-6.0% of the TRR, and the leaf PES fractions accounted for 14.0-26.6% of the TRR. Enzymatic digestion of the PES fractions from 14 DAT released an additional 6.9-14.0% of the TRR, and extraction with 1M NaOH from the remaining PES of [^{14}C-PH]-treated leaves released another 5.7% of the TRR. The overall recovery of radioactivity from the leaf samples was 97.1-100.1%.

HPLC and TLC analyses of the surface rinses and peel extracts identified ~90% of the TRR in/on fruits at 0 DAT and 58.3-65.1% of the TRR in/on fruits at 14 DAT. The metabolite profile in/on fruits was similar for both ^{14}C-labels. Acequinocyl was the major ^{14}C-residue in/on fruits at all sampling intervals, accounting for 81.6-83.7% of the TRR at 0 DAT, 46.2-58.4% of the TRR at 7 DAT, and 50.5-55.1% of the TRR by 14 DAT. Metabolite R1 was detected at 5.3-5.8% of the TRR at 0 DAT, and accounted for 4.3-10.3% of the TRR at 7 and 14 DAT. Trace amounts of AKM-18 (0.3-2.9% TRR) were also identified at each interval. The remaining solubilized ^{14}C-

residues from fruits were comprised primarily of minor polar metabolites, each accounting for <5% of the TRR. The unknown polar residues increased from 4.7-5.4% of the TRR on 0 DAT to 23.3-24.2% of the TRR by 14 DAT. In the [^{14}C-PH]-treated fruit, one of these polar metabolites at 14 DAT was identified as phthalic acid (1.7% TRR).

The metabolite profile in leaves was similar to fruits, although the percentage of polar metabolites was higher at the final sampling interval for leaves. Acequinocyl was the major ^{14}C-residue in/on leaves, accounting for 89.0-92.1% of the TRR at 0 DAT, 51.8-74.9% of the TRR at 7 DAT, and 38.4-58.1% of the TRR by 14 DAT. At all three intervals, Metabolite R1 accounted for 1.0-4.1% of the TRR and AKM-18 accounted for 0.3-6.7% of the TRR. Residues of phthalic acid were not quantified in leaves, but were identified as a component of the polar ^{14}C-residues. Unidentified, extractable ^{14}C-residues were comprised primarily of minor polar metabolites, each accounting for <10% of the TRR.

In the additional work conducted on the unknown polar ^{14}C-residues, the polar ^{14}C-residues were separated into three major regions, 1A, 1B, and 1C. Unknown 1C was shown to consist of phthalic acid by HPLC and TLC analyses of both the non-derivatized component and the methylated fraction. Treatment with either acid (2M HCl) or various hydrolytic enzymes (cellulase, hemicellulase, β-glucosidase, xylosidase, pectinase, and protease) had no appreciable affect on the proportion of these three components. However, treatment with base (2M) appeared to result in the degradation of 1A and 1B to 1C. Based on their partitioning behavior at various pHs, fractions 1A and 1B were characterized as being acidic in nature.

C.1. Storage Stability

Samples of eggplant leaves and fruits from the main study were collected during 8/94. Apparently, the samples were immediately surface washed after collection, but the dates of the sample extractions and analyses were not provided. The report stated that all samples and sample fractions were stored at <-15 C.

The only storage stability data provided in the report were from the repeated HPLC analyses of surface washes from 14 DAT fruit samples (both ^{14}C-labels) on two dates and from two TLC analyses of 14 DAT peel and leaf extracts on two dates. The provided HPLC chromatograms indicate that surface washes from fruits were initially profiled within ~1 month of collection. The subsequent reanalysis of these fractions 145 days later (4.5 months) indicates that residues of parent, which was the principle component, were stable in frozen storage. The TLC analyses of the peel and leaf extracts suggest that these extracts were initially profiled after 222 days (7.3 months) of frozen storage. The initial analyses show parent and three polar metabolites; however, the reanalysis 139-152 days later (4.5-5 months) shows that there was substantial loss/changes in the polar residues.

Considering that (i) parent was the major ^{14}C-residue (38.4-92.1% TRR) in/on fruit and leaves and was apparently stable in the surface washes and extracts over the course of analysis; (ii) the polar components which showed changes during storage were minor components (<10% TRR) of the ^{14}C-residue; and (iii) the additional work done on characterizing the polar residues utilized newly generated samples, no additional sample storage information or stability data will be required to support this metabolism study.

TABLE C.1. Summary of Storage Conditions

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Study Duration (days or months)	Limit of Demonstrated Storage Stability (days or months)
Fruits (surface rinses and peel and flesh extracts)	<-15 C	not provided	Parent was apparently stable in surface washes and extracts for ~5 months.
Leaves (surface rinses and leaf extracts)			

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1.1 TRR in/on Eggplant Fruits and Leaves Following a Single Foliar Application of [^{14}C-PH] or [^{14}C-DOD]-Acequinocyl at 0.51 and 0.55 lb ai/A, Respectively. [1]

Sample	Sampling interval (DAT)	Fraction	[^{14}C-PH]-Acequinocyl		[^{14}C-DOD]-Acequinocyl	
			% TRR	ppm	% TRR	ppm
Fruit	0	Surface wash	95.1	0.111	97.9	0.366
		Peel [2]	4.3	0.005	1.6	0.006
		Flesh [2]	0.5	<0.001	0.5	0.002
		Total	NA	0.116	NA	0.374
	7	Surface wash	70.1	0.061	51.3	0.080
		Peel [2]	21.8	0.019	44.2	0.069
		Flesh [2]	6.9	0.006	4.5	0.007
		Total	NA	0.087	NA	0.156
	14	Surface wash	60.7	0.085	78.7	0.048
		Peel [2]	28.6	0.040	18.0	0.011
		Flesh [2]	11.4	0.016	6.6	0.004
		Total	NA	0.140	NA	0.061
Leaves	0	Surface wash	97.9	13.68	98.2	26.11
		Leaves [2]	2.2	0.029	1.9	0.50
		Total	NA	13.98	NA	26.60
	7	Surface wash	69.5	15.70	89.0	11.60
		Leaves [2]	30.7	6.93	11.0	1.44
		Total	NA	22.60	NA	13.04
	14	Surface wash	67.8	4.89	79.8	3.99
		Leaves [3]	32.0	2.31	20.0	1.00
		Total	NA	7.21	NA	5.00

[1] Data are the average of 3 or 5 fruits and 3-5 leaves at each interval.
[2] Sum of radioactivity in extracts and residual solids.
NA = not applicable.

TABLE C.2.1.2 TRR in/on Leaves and Covered Fruits Following a Single Foliar Application of [^{14}C-PH] or [^{14}C-DOD]-Acequinocyl at 0.51 and 0.55 lb ai/A, Respectively. [1]

Sample	Sampling interval (DAT)	Fraction	[^{14}C-PH]-Acequinocyl		[^{14}C-DOD]-Acequinocyl	
			% TRR	ppm	% TRR	ppm
Fruit	7	Surface wash	NR	<0.001	NR	0.003
		Peel [2]	NR	0.001	NR	0.002
		Flesh [2]	NR	0.003	NR	0.001
		Total	NA	0.003	NA	0.004
	14	Surface wash	NR	0.006	NR	0.001
		Peel [2]	NR	0.006	NR	0.002
		Flesh [2]	NR	0.020	NR	0.003
		Total	NA	0.031	NA	0.005
Leaves	0	Surface wash	95.5	9.80	97.8	21.93
		Leaves [2]	4.4	0.45	2.0	0.047
		Total	NA	10.26	NA	22.42
	7	Surface wash	81.8	10.99	80.0	39.50
		Leaves [2]	18.2	2.45	19.9	9.81
		Total	NA	13.44	NA	49.32
	14	Surface wash	71.3	4.28	77.1	8.60
		Leaves [2]	28.6	1.72	22.9	2.56
		Total	NA	6.00	NA	11.15

[1] Data are the average of 2-5 fruits and 3 or 4 leaves at each interval.
[2] Sum of radioactivity in extracts and residual solids.
NR = not reported. Percentage distribution was not calculate due to low TRR levels.
NA = not applicable.

TABLE C.2.1.3 TRR in/on Leaves and Fruits Following a Single Soil Application of [^{14}C-PH] or [^{14}C-DOD]-Acequinocyl at 0.49 and 0.53 lb ai/A, Respectively. [1]

Sample	Sampling interval (DAT)	Fraction	[^{14}C-PH]-Acequinocyl	[^{14}C-DOD]-Acequinocyl
			ppm	ppm
Soil	0	0-5 cm	2.74	4.57
	14		2.13	3.41
Fruit	14	Surface wash	<0.001	<0.001
		Peel [2]	0.005	0.003
		Flesh [2]	0.007	0.004
		Total	0.012	0.005
Leaves	14	Surface wash	<0.001	<0.001
		Leaves [2]	0.018	0.031
		Total	0.018	0.031

[1] Data are the average of 4 fruit and 10 leaf samples.
[2] Sum of radioactivity in extracts and residual solids.

TABLE C.2.1.4 TRR in/on Leaves Fruits Following a Single Foliar Application of [^{14}C-PH]-Acequinocyl at 0.44 and 2.99 lb ai/A, Respectively. [1]

Sample	Sampling interval (DAT)	Fraction	Low Rate		High Rate	
			%TRR	ppm	%TRR	ppm
Leaves	14	Surface wash	82.8	3.00	87.2	21.66
		Leaves [2]	17.2	0.85	12.9	4.50
		Total	NA	3.85	NA	26.16
	28	Surface wash	69.1	2.12	72.2	14.9
		Leaves [2]	30.9	1.88	27.8	9.03
		Total	NA	4.00	NA	23.93

[1] Data are from pooled leaves samples at each interval (Supplemental Study).
[2] Sum of radioactivity in extracts and residual solids.
NA = not applicable.

TABLE C.2.2.1. Distribution and Characterization of ^{14}C-Residues in Eggplant Fruits Following a Single Foliar Application of [^{14}C-PH]Acequinocyl at 0.51 lb ai/A. [1]

Fraction/ Metabolites	Day 0 (TRR = 0.116 ppm)		Day 7 (TRR = 0.087 ppm)		Day 14 (TRR = 0.140 ppm)	
	%TRR	ppm	% TRR	ppm	%TRR	ppm
ACN surface rinse (HPLC)	95.7	0.111	70.1	0.061	60.7	0.085
Acequinocyl	81.6	0.095	40.1	0.035	44.8	0.063
R1	5.3	0.006	3.5	0.003	1.9	0.003
AKM-18	2.9	0.003	1.2	0.001	1.5	0.002
Minor HPLC unknowns (each <3% TRR)	5.4	0.006	7.7	0.007	10.2	0.014
Peel Extract (HPLC)	3.4 [2]	0.004	16.1	0.014	19.3	0.027
Acequinocyl			6.1	0.005	5.7	0.008
R1			2.2	0.002	2.4	0.003
AKM-18			0.2	<0.001	0.3	<0.001
Phthalic acid [3]			ND	--	1.7	0.002
Minor HPLC unknowns (each <5% TRR)			4.4	0.003	8.6	0.012
Flesh extract [2]	0.1	<0.001	2.2	0.002	3.6	0.005
Peel residual solids	0.7	0.001	5.7	0.005	9.3	0.013
Enzyme extract [4] (TLC)	NA	--	NA	--	6.1	0.007
5 Minor unknowns (each <2% TRR)					4.5	0.006
Residual solids	NA	--	NA	--	4.3	0.006
Flesh residual solids	0.4	<0.001	4.6	0.004	7.9	0.011

[1] Data are the average of 3 or 5 samples at each interval.
[2] The peel extract from Day 0 and each of the flesh extracts were not analyzed chromatographically due to low ^{14}C-residue levels.
[3] Phthalic acid was identified by TLC analyses of the polar fraction from HPLC analysis.
[4] Solids were incubated with cellulase, hemicellulase, pectinase, and β-glucosidase at pH 5 for 37 C, and were then extracted with ACN.

NA = not applicable.
ND = not detected.

TABLE C.2.2.2. Distribution and Characterization of ^{14}C-Residues in Eggplant Fruits Following a Single Foliar Application of [^{14}C-DOD]Acequinocyl at 0.55 lb ai/A. [1]

Fraction/ Metabolites	Day 0 (TRR = 0.374 ppm)		Day 7 (TRR = 0.156 ppm)		Day 14 (TRR = 0.061 ppm)	
	%TRR	ppm	% TRR	ppm	%TRR	ppm
ACN surface rinse (HPLC)	97.9	0.366	51.3	0.080	78.7	0.048
Acequinocyl	83.7	0.313	42.9	0.067	52.5	0.032
R1	5.8	0.022	0.4	0.001	1.1	0.001
AKM-18	0.3	0.001	1.9	0.003	<0.1	<0.001
Minor HPLC unknowns (each <2% TRR)	4.7	0.018	4.4	0.007	6.5	0.004
Peel Extract (HPLC)	1.3 [2]	0.005	35.9	0.056	11.5	0.007
Acequinocyl			15.5	0.024	2.6	0.002
R1			9.9	0.015	8.9	0.005
AKM-18			0.4	0.001	<0.3	<0.001
Minor HPLC unknowns (each <3% TRR)			8.7	0.014	13.4	0.008
Flesh extract [2]	0.3	0.001	2.6	0.004	3.3	0.002
Peel residual solids	0.3	0.001	8.3	0.013	6.6	0.004
Enzyme extract [3] (TLC)	NA	--	5.4	0.007	4.8	0.002
3 or 4 Minor unknowns (each <2% TRR)			3.9	0.006	4.3	0.003
Residual solids	NA	--	4.6	0.006	3.3	0.002
Flesh residual solids	0.3	0.001	1.9	0.003	3.3	0.002

[1] Data are the average of 3-5 samples at each interval.

[2] The peel extract from Day 0 and each of the flesh extracts were not analyzed chromatographically due to low ^{14}C-residue levels.

[3] Solids were incubated with cellulase, hemicellulase, pectinase, and β-glucosidase at pH 5 for 37 C, and were then extracted with ACN.

NA = not applicable.

TABLE C.2.2.3. Distribution and Characterization of ^{14}C-Residues in Eggplant Leaves Following a Single Foliar Application of [^{14}C-PH] Acequinocyl at 0.51 lb ai/A. [1]

Fraction/ Metabolites	Day 0 (TRR =13.98 ppm)		Day 7 (TRR = 22.60 ppm)		Day 14 (TRR = 7.21 ppm)	
	%TRR	ppm	% TRR	ppm	%TRR	ppm
ACN surface rinse (HPLC)	97.9	13.68	69.5	15.70	67.8	4.89
Acequinocyl	89.0	12.44	50.2	11.35	37.7	2.718
R1	4.1	0.573	3.0	0.633	2.1	0.151
AKM-18	0.3	0.042	2.0	0.452	6.0	0.433
Minor HPLC unknowns (each <10% TRR)	4.8	0.67	15.4 [3]	3.48	21.4 [3]	1.54
Leaf Extract (HPLC)	1.8 [2]	0.25	9.2	2.07	5.4	0.39
Acequinocyl			1.6	0.362	0.7	0.050
R1			0.3	0.068	0.3	0.022
AKM-18			0.3	0.068	0.7	0.050
Minor HPLC unknowns (each ≤4% TRR)			7.1	1.60	5.8	0.42
Residual solids	0.3	0.04	21.5	4.86	26.6	1.92
Enzyme extract [4] (TLC)	NA	--	11.4	2.69	16.8	1.27
5 Minor Polar unknowns (each <10% TRR)			9.2 [5]	2.06	14.0 [5]	1.01
1M NaOH extract (TLC)	NA	--	5.7	1.34	5.9	0.44
4 or 5 Minor polar unknowns (each <2% TRR)			4.4	0.99	4.3	0.31
Residual Solids			3.5	0.83	2.8	0.21

[1] Data are the average of 3-5 samples at each interval.
[2] The leaf extract from Day 0 was not analyzed chromatographically due to low ^{14}C-residue levels.
[3] Includes a polar fraction that accounted for 7.5 and 10.3% of the TRR in the 7 and 14 DAT samples. TLC analyses of this fraction separated fraction into two components; the larger component (6.9-9.7% TRR) was shown to include phthalic acid in the supplemental study.
[4] Solids were incubated with cellulase, hemicellulase, pectinase, and β-glucosidase at pH 5 for 37 C, and were then extracted with ACN.
[5] The largest fraction of radioactivity (6.2 and 9.7% TRR) from the TLC analyses was associated with a region (1B/C) that includes phthalic acid.
NA = not applicable.

TABLE C.2.2.4. Distribution and Characterization of ^{14}C-Residues in Eggplant Leaves Following a Single Foliar Application of [^{14}C-DOD]Acequinocyl at 0.55 lb ai/A. [1]

Fraction/ Metabolites	Day 0 (TRR =26.60 ppm)		Day 7 (TRR = 13.04 ppm)		Day 14 (TRR = 5.00 ppm)	
	%TRR	ppm	% TRR	ppm	%TRR	ppm
ACN surface rinse (HPLC)	98.2	26.11	89.0	11.60	79.8	3.99
Acequinocyl	92.1	24.50	74.9	9.77	58.1	2.91
R1	2.1	0.559	2.2	0.287	1.0	0.050
AKM-18	0.3	0.080	1.1	0.143	3.1	0.155
Minor HPLC unknowns (each <5% TRR)	3.9	1.04	10.0	1.30	18.2	0.91
Leaf Extract [2]	1.7	0.46	6.6	0.86	6.0	0.30
Residual solids	0.2	0.04	4.4	0.58	14.0	0.70
Enzyme extract [3] (TLC)	NA	--	NA	--	6.9	0.36
5 Minor Polar unknowns (each <3% TRR)					6.4	0.32
Residual Solids					6.4	0.34

[1] Data are the average of 3-5 samples at each interval.
[2] The leaf extracts were not analyzed chromatographically due to low ^{14}C-residue levels.
[3] Solids were incubated with cellulase, hemicellulase, pectinase, and β-glucosidase at pH 5 for 37 C, and were then extracted with ACN.
NA = not applicable.

Table C.2.3.1. Summary of Characterization and Identification of ^{14}C-Residues in/on Eggplant Fruits Following a Single Foliar Application of [^{14}C-PH]- or [^{14}C-DOD]-Acequinocyl ~0.5 lb ai/A. [1]

[^{14}C-PH]-Acequinocyl						
Metabolites/Fractions [2]	Day 0 (TRR = 0.116 ppm)		Day 7 (TRR = 0.087 ppm)		Day 14 (TRR = 0.140 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Acequinocyl	81.6	0.095	46.2	0.040	50.5	0.071
Metabolite R1	5.3	0.006	5.7	0.005	4.3	0.006
AKM-18	2.9	0.003	1.4	0.001	1.8	0.002
Phthalic acid	ND	--	ND	--	1.7	0.002
Total identified	**89.8**	**0.104**	**53.3**	**0.046**	**58.3**	**0.081**
Minor Unknowns (each ≤5%TRR)	5.4	0.006	12.1	0.010	23.3	0.032
Minor solvent fractions	3.5	0.004	2.2	0.002	3.6	0.005
Total characterized	**98.7**	**0.114**	**67.6**	**0.058**	**85.2**	**0.118**
Total extractable [4]	99.2	0.115	88.4	0.077	89.7	0.126
Total bound [5]	1.1	0.001	10.3	0.009	12.2	0.017
Accountability (% TRR recovered)	100.3		98.7		101.9	
[^{14}C-DOD]-Acequinocyl						
Metabolites/Fractions [2]	Day 0 (TRR = 0.374 ppm)		Day 7 (TRR = 0.156 ppm)		Day 14 (TRR = 0.061 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Acequinocyl	83.7	0.313	58.4	0.091	55.1	0.034
Metabolite R1	5.8	0.022	10.3	0.016	10.0	0.006
AKM-18	0.3	0.001	2.3	0.004	<1.0	<0.001
Total identified	**89.8**	**0.336**	**71.0**	**0.111**	**65.1**	**0.040**
Minor Unknowns (each <3%TRR)	4.7	0.018	17.0	0.027	24.2	0.015
Minor solvent fractions	1.6	0.006	2.6	0.004	3.3	0.002
Total characterized	**96.1**	**0.360**	**90.6**	**0.142**	**92.6**	**0.057**
Total extractable [4]	99.5	0.372	95.2	0.149	98.3	0.059
Total bound [5]	0.6	0.002	6.5	0.009	6.6	0.004
Accountability (% TRR recovered)	100.1		101.7		104.9	

[1] For each interval, the data are the average of 3-5 samples, which were analyzed individually. ^{14}C-Residues were quantified by HPLC, except for phthalic acid, which was quantified by TLC.
[2] The chemical names and structures for acequinocyl and its metabolites are presented in Table C.3.1.
[3] Phthalic acid was identified (TLC) as a component of the polar residues from the peel extract (14 DAT sample).
[4] Total extractable includes ^{14}C-released by solvent extraction and enzyme digestion.
[5] Total bounds radioactivity includes residual solids from peel and flesh.
ND = not detected.

Table C.2.3.2. Summary of Characterization and Identification of ^{14}C-Residues in/on Eggplant Leaves Following a Single Foliar Application of [^{14}C-PH]- or [^{14}C-DOD]-Acequinocyl ~0.5 lb ai/A. [1]

[^{14}C-PH]-Acequinocyl						
Metabolites/Fractions [2]	Day 0 (TRR =13.98 ppm)		Day 7 (TRR = 22.60 ppm)		Day 14 (TRR = 7.21 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Acequinocyl	89.0	12.44	51.8	11.71	38.4	2.79
Metabolite R1	4.1	0.573	3.3	0.70	2.4	0.17
AKM-18	0.3	0.042	2.3	0.52	6.7	0.49
Total identified	**93.4**	**13.06**	**57.4**	**12.93**	**47.5**	**3.45**
Minor Unknowns (each <10%TRR)	4.8	0.67	36.1 [3]	8.16	45.5 [3]	3.28
Minor solvent fractions	1.8	0.25	NA	--	NA	--
Total characterized	**100.0**	**13.98**	**93.5**	**21.09**	**93.0**	**6.73**
Total extractable [4]	99.7	13.93	95.8	21.80	94.3	6.80
Total bound	0.3	0.04	3.5	0.83	2.8	0.21
Accountability (% TRR recovered)	100.0		99.3		97.1	
[^{14}C-DOD]-Acequinocyl						
Metabolites/Fractions [2]	Day 0 (TRR =26.60 ppm)		Day 7 (TRR = 13.04 ppm)		Day 14 (TRR = 5.00 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Acequinocyl	92.1	24.50	74.9	9.77	58.1	2.91
Metabolite R1	2.1	0.56	2.2	0.29	1.0	0.05
AKM-18	0.3	0.08	1.1	0.14	3.1	0.16
Total identified	**94.5**	**25.14**	**78.2**	**10.20**	**62.2**	**3.12**
Minor Unknowns (each <5%TRR)	3.9	1.04	10.0	1.30	24.6	1.23
Minor solvent fractions	1.7	0.46	6.6	0.86	6.0	0.30
Total characterized	**100.1**	**26.64**	**94.8**	**12.36**	**92.8**	**4.65**
Total extractable [4]	99.9	26.57	95.6	12.46	92.7	4.65
Total bound	0.2	0.04	4.4	0.58	6.4	0.34
Accountability (% TRR recovered)	100.1		100.0		99.1	

[1] For each interval, the data are the average of 3-5 samples, which were analyzed individually. ^{14}C-Residues were quantified by HPLC, except for polar ^{14}C-residues, which was quantified by TLC.

[2] The chemical names and structures for acequinocyl and its metabolites are presented in Table C.3.1.

[3] The major unknown components consisted for polar fractions in the surface wash and enzyme extracts that were associated with TLC region 1B/C (6.2-9.7% TRR). In the supplemental study, analysis of polar ^{14}C-residues identified TLC region 1C as phthalic acid.

[4] Total extractable includes radioactivity released by solvent extraction, enzyme digestion, and base extraction (1M NaOH).

NA = not applicable.

C.3. Proposed Metabolic Profile

FIGURE C.3.1. Proposed Metabolic Profile of Acequinocyl in Eggplant.

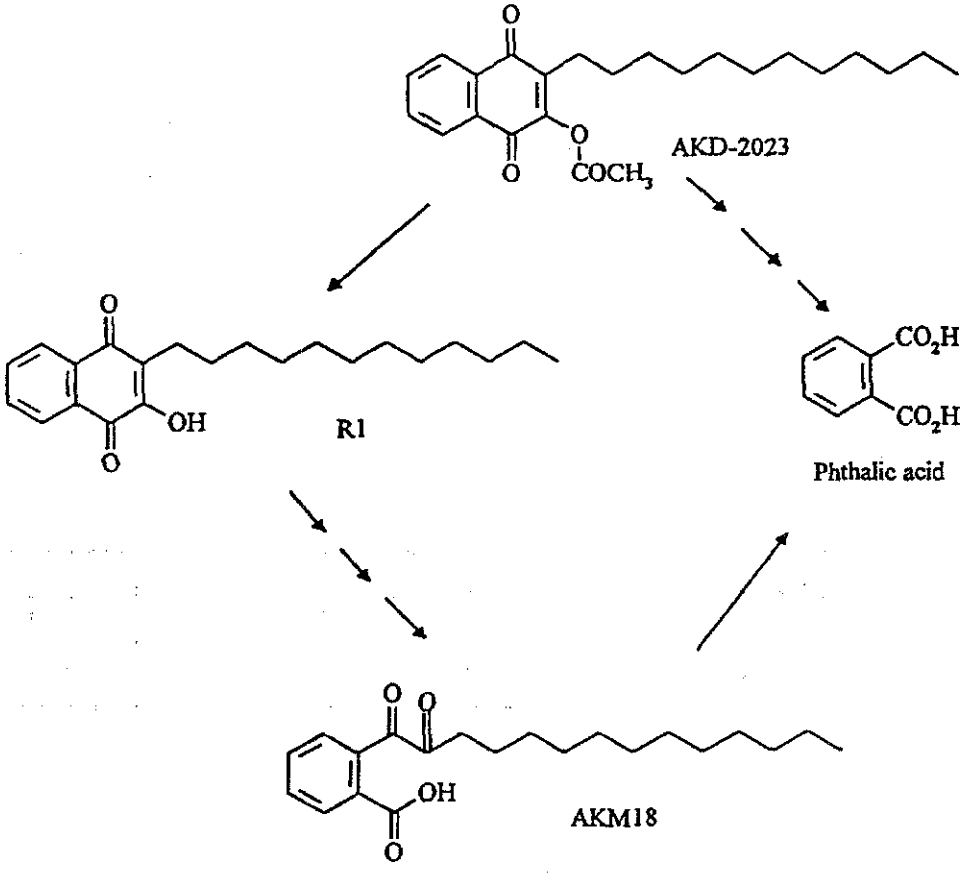
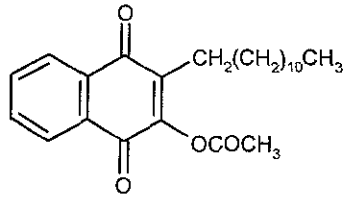
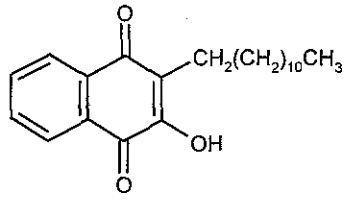
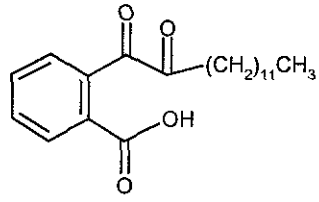
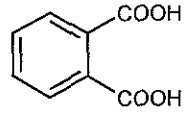


TABLE C.3.1. Identification of Compounds in Eggplant Metabolism Study.

Common name/code	Chemical name	Chemical structure
Acequinocyl AKD-2023	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione	
Acequinocyl-OH (Metabolite R1)	2-dodecyl-3-hydroxy-1,4-naphthalenedione	
AKM-18	2-(1,2-dioxotetradecyl)-benzoic acid	
phthalic acid		

D. CONCLUSION

The [^{14}C]-acequinocyl eggplant metabolism study is adequate. Parent was the major ^{14}C-residue identified in/on fruits and leaves at each sampling interval, accounting for 50.5-55.1% of the TRR in/on fruit and 38.4-58.1% of the TRR in/on leaves by 14 DAT. Metabolite R1 accounted for 4.3-10.3% of the TRR in fruit and 1.0-4.1% of the TRR in leaves, and Metabolite AKM-18 accounted for 0.3-2.9% of the TRR in fruit and 0.3-6.7% of the TRR in leaves. Minor amounts of phthalic acid were also identified in fruit and leaves. The remaining residues were comprised of minor unknown components that were primarily polar in nature. Based on the identified metabolites, the metabolism of acequinocyl in eggplant appears to involve the loss of the acetyloxy moiety to form Metabolite R1, opening of the quinone ring to form AKM-18, and subsequent degradation of the quinone ring to yield phthalic acid.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (~~HED~~; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 20-NOV-2002). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651704 Corden, M. (1999) [^{14}C-Phenyl] AKD 2023 Metabolism in the Lactating Goat: Lab Project Number: AGK/049. Unpublished study prepared by Huntingdon Life Sciences Ltd. 70 pages.

EXECUTIVE SUMMARY:

In a ruminant metabolism study, a single dairy goat (*capra hircus*, British Saaneen) was dosed orally, via capsules, once a day for 5 consecutive days with [U-^{14}C-phenyl]-acequinocyl (>96.7% radiochemical purity) at 17.66 mg/day, equivalent to 0.28 mg/body weight/day. Based on actual feed consumption, this dose level was equivalent to 11.3 ppm of [^{14}C]acequinocyl in the diet. Although the goat used in the study was dosed at the minimum level recommended by the Agency, the resulting levels of radioactivity in milk and tissues were too low to provide adequate identification of the ^{14}C-residues.

A total of 85.7% of the administered dose was recovered, with the majority of the dose being recovered in the feces (64.2% dose) and G.I. tract (10.8% dose). Another 9.9% of the administered dose was recovered in the urine. Radioactivity remaining in edible tissues at sacrifice accounted for 0.7% of the dose and <0.1% of the dose was excreted in the milk. Maximum levels of radioactivity in milk were 0.0027 ppm (Day 5), and total radioactive residues (TRR) in tissues were relatively low at 0.14 ppm in liver, 0.10 ppm in kidneys, 0.017-0.018 ppm in fat, and 0.006-0.008 ppm in muscle.

Solvent extraction released 55.7-77.5% of the TRR from liver, kidney and fat, and protease digestion released an additional 12.9-23.9% of the TRR from kidneys and liver. Radioactivity remaining in the residual solids from each tissue accounted for <0.03 ppm, and the overall recovery of radioactivity from tissues was ~100%.

Characterization of ^{14}C-residues in liver, kidneys and fat was limited, and quantitation of ^{14}C-residues was problematic due to poor separation of components. In liver, parent (1.5% TRR) was detected along with Metabolites R1 (8.4% TRR), AKM-15 (9.0% TRR), and AKM-18 (1.8% TRR). Parent (10.3% TRR) was also detected in kidneys along with Metabolites R1 (<1%

TRR), AKM-15 (9.1% TRR), and AKM-18 (6.2% TRR). In fat, parent and Metabolite R1 (unresolved) together accounted for 44.0% of the TRR. Supporting analyses of feces and urine were also limited, but tentatively detected parent (14.4%) and Metabolites R1 (9.3%) and AKM-18 (27.1%) in feces and phthalic acid (33-42%) in urine. With the exception of AKM-15 in liver, the identity of parent and the remaining metabolites were not adequately confirmed.

Based on the limited data available, the metabolism of acequinocyl in goats appears to involve loss of the acetyloxy moiety to form Metabolite R1 (also referred to as acequinocyl-OH) and partial cleavage of the dodecyl side chain to form AKM-15. Opening and degradation of the quinone ring was also evidenced by the presence of AKM-18 and phthalic acid.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

This ruminant metabolism study is classified as scientifically acceptable. The acceptability of this study for regulatory purposes is also addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance and Data Confidentiality statements were provided.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. These uses could result in the transfer of residues to livestock by the feeding of wet apple pomace, dried citrus pulp, and/or almond hulls. There are currently no food/feed uses or tolerances for acequinocyl.

TABLE A.1. Test Compound Nomenclature

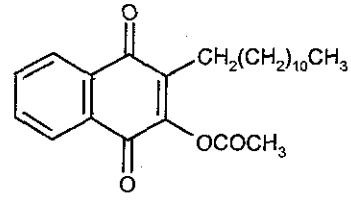
Compound	Chemical Structure
	
Common name	Acequinocyl
Company experimental name	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use product/EP	Kanemite™ 15 SC, 1.25 lb/gal FIC

TABLE A.2. Physicochemical Properties

Parameter	Value	Reference (MRID#)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20˚C)	6.69 µg/L	45434906
Solvent solubility (mg/L at 20˚C)	1,2-dichloroethane: >250 acetone: >250 ethyl acetate: >250 xylene: >250 methanol: 6.1 n-heptane: 36 n-octanol: 29.2	45434904
Vapor pressure at 25˚C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK$_a$)	no measurable pK$_a$	45434905
Octanol/water partition coefficient Log(K$_{OW}$)	≥6.2	45434906
UV/visible absorption spectrum	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Livestock

The in-life and analytical phases of the study were conducted by Huntingdon Life Sciences (Cambridgeshire, England), with dosing occurring from 12/17-12/21/97. A single dairy goat was dosed orally, once a day, for 5 consecutive days with [U-^{14}C-phenyl]-acequinocyl at a dose of 17.66 mg ai/day, equivalent to 0.28 mg/kg bw/day. The test animal was observed daily for general health and appearance during the dosing period and was sacrificed within 23 hours of receiving the final dose. No difficulties were noted during dosing and no adverse reactions were observed following dosing.

TABLE B.1.1. General Test Animal Information

Species	Breed	Age	Weight at study initiation (kg)	Health Status	Description of housing/ holding area
Dairy goat (*Capra hircus*)	British Saanen	2-5 years	61.5 kg	good health	stainless steel metabolism cage Temperature: 14-22° C (no other environmental data were provided)

TABLE B.1.2. Test Animal Dietary Regime

Diet [1]	Water	Acclimation period	Predosing
Mixture ration (Meadow Fresh Goat Mix) and Listers Grass Nuts	*ad libitum*	3 days	None

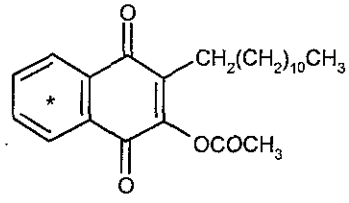
[1] Food was offered twice a day, following milking.

TABLE B.1.3. Test Animal Dosing Regime

Treatment Type	Administered dose (mg/day)	Average Food consumption (kg/day)	Dietary level [1] (ppm)	Vehicle	Timing [2]/Duration
Oral	17.66	1.567	11.3	gelatin capsules with glucose	Once a day for 5 days

[1] Based on actual feed consumption.
[2] Goat was dose following the morning milking.

B.2. Test Materials

TABLE B.2.1. Test Material Characteristics	
Chemical structure	 * indicates ^{14}C-position
Radiolabel position	Uniformly ^{14}C-labeled in the phenyl ring
Lot No.	CP-1997
Radiochemical Purity	≥96.7% [1]
Specific activity	2.36 MBq/mg (1.64 MBq/mg; 98,124 dpm/μg) [2]
Code	[^{14}C-PH]

[1] Determined by TLC analysis of prepared dosing capsules.
[2] Specific activity of the final dosed ^{14}C-material.

B.3. Sampling Information

Urine, feces, and cage washes were collected daily immediately before and during the dosing period. Samples were immediately frozen. Milk was collected twice a day in the morning and afternoon and was refrigerated until radioassay and was then frozen. Blood samples were collected at 1, 2, 3, 4, 6, 8, 12, and 23 hours following the first and last doses and 12 hours after dosing on Study Days 2, 3, and 4. These blood samples were centrifuged to obtain plasma and were radioassayed. The goat was sacrificed ~23 hours following the final dose and the samples listed in Table B.3.1 were collected. Samples were stored frozen (<-15 C) following collection.

TABLE B.3.1. Sample Collection Information					
Milk collection	Milk production [1] (ml/day)	Excreta collected	Interval from last dose to sacrifice	Samples collected at sacrifice and analyzed	
Twice a day	966	Urine, feces and Cage wash (daily)	~23 hours	foreleg muscle subcutaneous fat [2] omental fat [2] kidney bile	rump muscle perirenal fat [2] liver blood G.I. tract and contents

[1] During Study Days 1-5.
[2] The fat samples were composited for analysis.

B.4. Identification/ Characterization of Residues

B.4.1. Sample Preparation, Radioassay, and Extraction

Samples of milk, urine, and bile were radioassayed in triplicate directly by liquid-scintillation counting (LSC). Homogenized feces (Day 1), tissue samples (except fat) and whole blood were radioassayed in triplicate by combustion and LSC. Homogenized fat samples were digested with a tissue solubilizer (NCS II) at 55 C for 18 hours and then radioassayed in triplicate by LSC. To determine radioactivity in feces from Study Days 2-5 and the G.I. tract and contents, samples were solvent extracted and radioactivity in the extracts (determined by LSC) and solids (determined by combustion/LSC) were summed. The limit of quantitation (LOQ) for the radioassays was not reported.

Radioactive residues in liver and kidney were sequentially extracted by homogenizing with acetonitrile (ACN), ACN:water (3:1, v/v) and methanol (MeOH, liver only), centrifuging after each extraction. The extracts were combined and concentrated to dryness, and the solubilized ^{14}C-residues were redissolved in ACN for high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) analyses. The post-extraction solids (PES) were then subjected to protease treatment in 1M TRIS buffer (pH 10) at 37 C for 48 hours. The protease hydrolysate was centrifuged, and radioactivity in the supernatant and residual solids were determined.

Radioactive residues in a composited fat sample were sequentially extracted by homogenizing with hexane, ACN, and ACN:water (3:1, v/v), centrifuging after each extraction. Radioactivity in the hexane fraction was partitioned into ACN, and the resulting ACN fraction was combined with the ACN and ACN:water extracts. The combined fraction was then concentrated to dryness, and ^{14}C-residues were redissolved in ACN for HPLC and TLC analyses. The PES fraction was radioassayed.

Separate urine samples from each day were analyzed directly by HPLC and TLC. A pooled (Study Days 2-5) sample of feces was extracted repeatedly with ACN, centrifuging after each extraction. The fecal extracts were combined, concentrated and analyzed by HPLC and TLC.

B.4.2. Analytical Methodology

Radioactive residues in solvent fractions were analyzed by reverse-phase HPLC and normal-phase 1D-TLC. Two HPLC systems were used for analysis. The primary HPLC system consisted of a C_8-column using a mobile phase gradient of aqueous ammonium formate (0.015 M) to ACN, with a ultraviolet (UV) detector. The secondary HPLC system, used primarily for analyzing polar metabolites, consisted of a PRP-1 column (Hamilton) using a mobile phase gradient of aqueous ammonium formate (0.01M) to ACN, with a UV detector. Radioactivity was determined by LSC of collected eluate fractions. The TLC analyses used silica gel plates with either chloroform:MeOH (9:1, v/v) or ACN:water (8:2, v/v) as the solvents. Including parent, a total of 22 reference standards were used for comparison. Standards were detected by UV absorbance (254 nm) for HPLC and by UV quenching on TLC plates. ^{14}C-Residues extracted

from tissues and feces were quantified using the HPLC data. Quantitative data for urine are from TLC analyses.

C. RESULTS AND DISCUSSION

A single lactating goat was dosed orally via capsules once a day for 5 days with [U-^{14}C-phenyl]-acequinocyl at an average dose of 17.66 mg/day or 0.28 mg/kg bw/day. Based on actual feed consumption, the average dose was equivalent to 11.3 ppm of [^{14}C]acequinocyl in the diet.

At sacrifice, a total of 85.7% of the administered dose was recovered, with the majority of the dose being recovered in the feces (64.2% dose) and G.I. tract (10.8% dose). Another 9.9% of the administered dose was recovered in the urine (Table C.2.1.1). After Study Day 1, the daily excretion of radioactivity in urine accounted for 1.9-2.4% of the dose, and the daily excretion of radioactivity in the feces accounted for 11.7-18.0% of the dose (Figure C.2.1). Radioactivity remaining in edible tissues at sacrifice accounted for 0.7% of the dose and <0.1% of the dose was excreted in the milk. Although radioactivity in milk was low (<0.01 ppm), ^{14}C-residues in milk increased throughout dosing to a maximum of 0.0027 ppm on the afternoon of Day 5. TRRs in tissues were relatively low at 0.14 ppm in liver, 0.10 ppm in kidneys, 0.017-0.018 ppm in fat, and 0.006-0.008 ppm in muscle. Following both the first and final doses, peak plasma concentrations (T_{max}) of radioactivity were observed at 12 hours post-dose. Concentrations in plasma at 12 hours post-dose were 0.060 ppm on Study Day 1, and were relatively steady at 0.093-0.104 ppm on Study Days 2 through 5 (Table C.2.1.2; Figure C.2.2).

As TRRs were <0.01 ppm in milk and muscle, these matrices were not extracted for analysis. For liver, kidneys, and fat samples, solvent extraction released 55.7-77.5% of the TRR (Table C.2.2). Protease digestion released an additional 12.9 and 23.9% of the TRR from kidneys and liver, respectively; however, these fractions were not further analyzed. Radioactivity remaining in the residual solids from each tissue accounted for <0.03 ppm. The overall recovery of radioactivity from liver, kidneys, and fat was ~100%.

Characterization of ^{14}C-residues in liver, kidneys and fat was limited, and quantitation of ^{14}C-residues was problematic due to poor separation of components. For liver, parent and the Metabolite R1 were detected by HPLC System 1 at 1.5% and 8.4% of the TRR, respectively, but were not resolved by the other HPLC system. Metabolites AKM-15 (9.0% TRR) and AKM-18 (1.8% TRR) were detected on HPLC System 2, and the presence of AKM-15 was confirmed by co-chromatography with the reference standard using normal-phase 1D-TLC. However, the identities of parent and the other two metabolites in liver were not confirmed by any other means. The situation with the kidney extract was similar. Parent (10.3% TRR) and Metabolites R1 (<1% TRR), AKM-15 (9.1% TRR), and AKM-18 (6.2% TRR) were each detected in kidney, but each component was resolved on only one of the HPLC systems and the data from the single 1D-TLC chromatogram were inconclusive. For fat, only a single HPLC analysis was provided that did not resolve parent from Metabolite R1. No chromatographic analyses were performed on liver or kidney protease fractions.

Supporting analyses of feces and urine were similarly limited. Quantitative data for metabolites in urine were obtained from the 1D-TLC analysis in which radioactivity essentially remained at or adjacent to the origin. HPLC analysis tentatively detected phthalic acid in urine, but no other confirmatory data were provided. HPLC analysis of the fecal extract detected parent (14.4% fecal radioactivity) and Metabolites R1 (9.3%) and AKM-18 (27.1%); however, no other analyses were conducted to confirm the identities of parent or these metabolites.

C.1. Storage Stability

Samples of apple leaves and fruits from the main study were immediately surface washed after collection. All samples and sample fractions were then stored at <-15 C.

The only storage stability data provided in the report were from the repeated HPLC analyses of surface washes from fruit samples (both ^{14}C-labels) on two dates and from two TLC analyses of peel extracts on two dates. The provided HPLC chromatograms indicate that surface washes from fruits were initially profiled within ~1 month of collection. The subsequent reanalysis of these fractions 3 months later indicates that residues of parent, which was the principle component, were stable in frozen storage. The TLC analyses of the peel extracts suggest that these extracts were initially profiled after ~3 months of frozen storage. The initial analyses show parent and two polar metabolites (1A and 1B); however, the reanalysis ~6 months later shows that there was substantial loss of one of the polar residues (Unknown 1B).

Considering that (i) parent was the major ^{14}C-residue (20.0-93.5% TRR) in/on fruit and leaves; (ii) parent was apparently stable in the fruit surface washes and extracts over the course of analysis; (iii) the polar components which showed changes during storage were minor components (<5% TRR) of the ^{14}C-residue; and (iv) the additional work done on characterizing the polar residues utilized newly generated samples, no additional sample storage information or stability data will be required to support this metabolism study.

TABLE C.1. Summary of Storage Conditions

Matrix (RAC or Extract)	Storage Temp. (°C)	Actual Study Duration (months)	Limit of Demonstrated Storage Stability (days or months)
Fruits (surface rinses and peel extracts)	<-15 C	1-8 [1]	Parent was apparently stable in surface washes and extracts for ~3 months.
Fruits (flesh extracts)		Not provided	
Leaves (surface rinses and leaf extracts)		1-2	

[1] Peel extracts from 30 DAT fruit treated with the C^{14}-DOD label were stored for 8 months, all others were stored 1-2 months.

C.2. Identification, Characterization, and Distribution of Residues

TABLE C.2.1.1 Total Radioactive Residues (TRRs) in Milk, Tissues, Urine, and Feces from a Goat Dosed Orally for 5 Consecutive Days with [U-phenyl-^{14}C]-Acequinocyl at 0.28 mg/kg bw/day, Equivalent to 11.3 ppm in the Diet.

Matrix	Study Day	Total radioactive residues	
		% Dose	ppm
Milk	1 (am)	NR	<0.0003
	1 (pm)	NR	0.0003
	2 (am	NR	0.0010
	2 (pm)	NR	0.0015
	3 (am)	NR	0.0015
	3 (pm)	NR	0.0016
	4 (am)	NR	0.0023
	4 (pm)	NR	0.0021
	5 (am)	NR	0.0019
	5 (pm)	NR	0.0027
	6 (am)	NR	0.0019
	Total	<0.1	NA
Bile	5	<0.1	1.31
Blood	5	0.2	0.040
Foreleg muscle	5	0.2	0.006
Rump muscle	5		0.008
Liver	5	0.2	0.140
Kidneys	5	<0.1	0.100
Subcutaneous fat	5	0.1	0.018
Perirenal fat	5		0.017
Omental fat	5		0.018
Total in edible tissues and blood	5	0.7	NA
G.I. tract and contents	5	10.8	NR
Urine	1	1.2	NR
	2	2.2	NR
	3	2.2	NR
	4	1.9	NR
	5	2.4	NR
	Total	9.9	NA
Cage wash	1	0.01	NR
	2	0.02	NR
	3	0.01	NR
	4	0.03	NR
	5	0.05	NR

TABLE C.2.1.1 Total Radioactive Residues (TRRs) in Milk, Tissues, Urine, and Feces from a Goat Dosed Orally for 5 Consecutive Days with [U-phenyl-^{14}C]-Acequinocyl at 0.28 mg/kg bw/day, Equivalent to 11.3 ppm in the Diet.

Matrix	Study Day	Total radioactive residues	
		% Dose	ppm
	Total	0.12	NA
Feces	1	2.9	NR
	2	11.7	NR
	3	18.0	NR
	4	17.6	NR
	5	14.0	NR
	Total	64.2	NA
Total recovery		85.7	NA

NA = not applicable.
NR = nor reported.

TABLE C.2.1.2 Concentration of Radioactivity in Plasma from a Goat Dosed Orally for 5 Consecutive Days with [U-phenyl-^{14}C]-Acequinocyl at 0.28 mg/kg bw/day, Equivalent to 11.3 ppm in the Diet.

Matrix	Sampling interval			Concentration of ^{14}C-Residues (ppm)
	Study day	Study hour	hours since last dose	
Plasma	1	1	1	0.001
		2	2	0.001
		3	3	0.004
		4	4	0.006
		6	6	0.019
		8	8	0.038
		12	12	0.060
		23	23	0.034
	2	36	12	0.093
	3	60	12	0.097
	4	84	12	0.104
	5	97	1	0.059
		98	2	0.061
		99	3	0.051
		100	4	0.058
		102	6	0.079
		104	8	0.085
		108	12	0.098
		119	23	0.055

FIGURE C.2.1. Excretion of Radioactivity in the Urine and Feces of a Lactating Goat Dosed for 5 Days with Acequinocyl at 0.28 mg/kg bw/day, Equivalent to 11.3 ppm in the Diet.

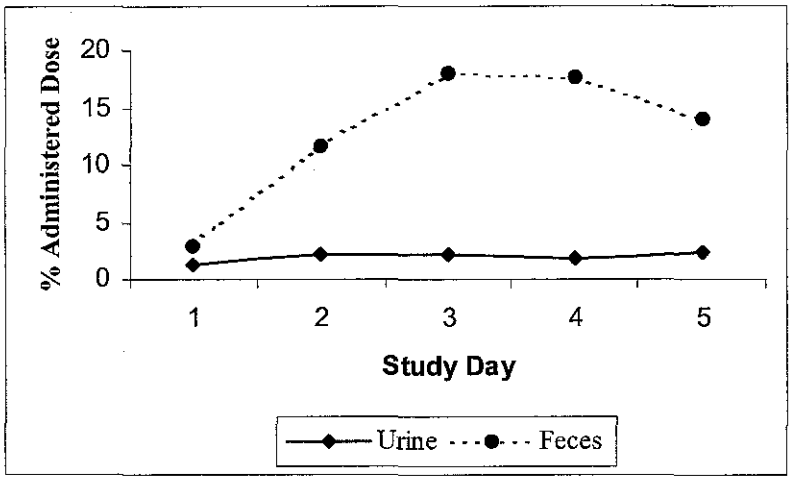
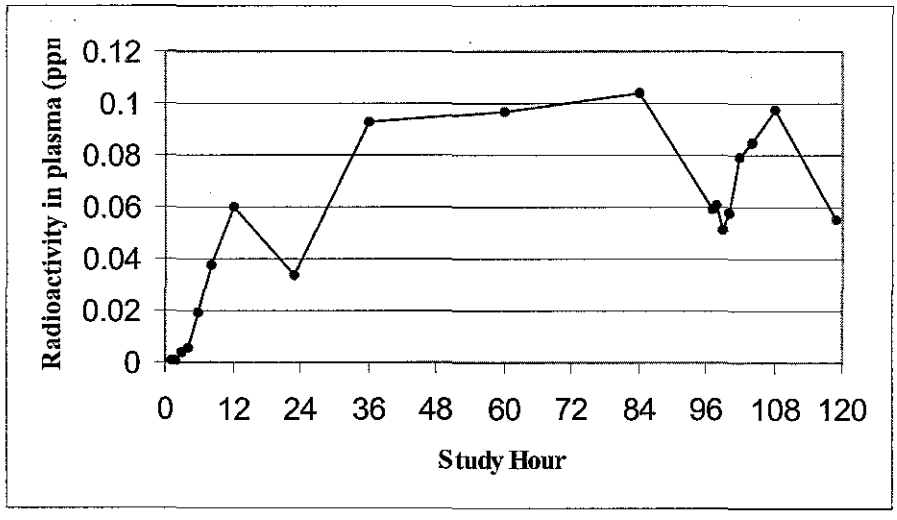


FIGURE C.2.2. Concentration of Radioactivity (ppm) in plasma Over 5 Days of Dosing with [^{14}C]Acequinocyl at 0.28 mg/kg bw/day [1].



[1] Goat was dosed at 0, 24, 48, 72 and 96 hours. Maximum plasma concentration of radioactivity occurred at 12 hours post-dose; Study Hours 12, 36, 60, 84 and 108.

TABLE C.2.2. Distribution of ^{14}C-Residues in Liver, Kidney, and Fat from a Goat Dosed for 5 Days with [^{14}C-phenyl]-Acequinocyl at 0.28 mg/kg bw/day, Equivalent to 11.3 ppm in the Diet.

Fraction/ Metabolite	Liver (TRR = 0.140 ppm)		Kidney (TRR = 0.100 ppm)		Fat (TRR = 0.018 ppm)	
	%TRR	ppm	% TRR	ppm	%TRR	ppm
Combined Solvent extracts [1]	55.7	0.078	77.5	0.078	75.4	0.014
ACN (HPLC analysis)	55.7	0.078	77.5	0.078	75.4	0.014
Acequinocyl/MetaboliteR 1	5.0 [2]	0.007	10.3 [3]	0.010	44.0	0008
Metabolite AKM-15	9.0	0.013	9.1	0.009	ND	--
Metabolite AKM-18	1.8	0.003	16.1[4]	0.016	ND	--
Major Polar Unknown	6.0	0.009	11.5	0.012	17.6	0.003
Minor unknowns (each ≤4.6% TRR)	14.6	0.022	19.0	0.021	ND	--
Unresolved radioactivity [5]	19.3	0.028	11.5	0.012	13.9	0.003
Protease hydrolysate	23.9	0.033	12.9	0.013	NA	--
Residual solids (PES)	20.4	0.029	9.6	0.010	24.6	0.004

[1] The various solvent extracts for each tissue were combined, concentrated to dryness, and redissolved in ACN for analysis.
[2] Analysis on a different HPLC system indicated that acequinocyl accounted for 1.5% of the TRR and Metabolite R1 accounted for 8.4% of the TRR.
[3] Analysis on a different HPLC system indicated that Metabolite R1 accounted for <1% of the TRR (<0.001 ppm).
[4] Separated into two components on a different HPLC system, with Metabolite AKM-18 accounting for 6.2% of the TRR (0.006 ppm)
[5] Radioactivity not associated with a specific peak.
NA = not applicable; ND = not detected.

Table C.2.3.1. Summary of the Characterization and Identification of ^{14}C-Residues in Liver, Kidney and Fat from a Goat Dosed Orally for 5 Days with [U-^{14}C-phenyl]-Acequinocyl at 17.66 mg/day, Equivalent to 11.3 ppm in the Diet.

Fraction/ Metabolite [1]	Liver (TRR = 0.140 ppm)		Kidney (TRR = 0.100 ppm)		Fat (TRR = 0.018 ppm)	
	% TRR	ppm	% TRR	ppm	% TRR	ppm
Acequinocyl	1.5	0.002	10.3	0.010	44.0 [2]	0.008
R1 (acequinocyl-OH)	8.4	0.012	<1.0	<0.001		
AKM-15 [3]	9.0	0.013	9.1	0.009	ND	--
AKM-18	1.8	0.003	6.2	0.006	ND	--
Total tentatively identified	**20.7**	**0.018**	**26.6**	**0.026**	**44.0**	**0.008**
Major polar unknown(s)	6.0	0.009	11.5	0.012	17.6	0.003
Minor Unknowns (<10% TRR)	14.6	0.022	28.9	0.029	NA	--
Unresolved radioactivity [4]	19.3	0.028	11.5	0.012	13.9	0.003
Protease solubilized	23.9	0.033	12.9	0.013	NA	--
Total characterized	**84.5**	**0.110**	**91.4**	**0.092**	**75.5**	**0.014**
Total extractable [5]	79.6	0.111	90.4	0.091	75.4	0.014
Residual solids	20.4	0.029	9.6	0.010	24.6	0.004
Accountability (% TRR recovered)	100.0		100.0		100.0	

ND = not detected; NA = not applicable.

[1] The chemical names and structures for acequinocyl and its putative metabolites are presented in Table C.3.1. With the exception of AKM-15 in liver extract, the identities of parent and the remaining metabolites are tentative.

[2] HPLC analysis did not resolve parent and metabolite R1 in the fat extract.

[3] The presence of AKM-15 in liver extracts was confirmed by TLC and HPLC analyses.

[4] Radioactivity from HPLC analyses that was not associated with a particular peak.

[5] Includes radioactivity released by protease treatment.

TABLE C.2.3.2 Summary of the Characterization and Identification of ^{14}C-Residues in Feces and Urine from a Goat Dosed Orally for 5 Days with [^{14}C-phenyl]-Acequinocyl at a Level Equivalent to 11.3 ppm in the Diet.

Fraction/ Metabolite [1]	% Total Radioactive Residue	
	Feces [2]	Urine [3]
Acequinocyl [4]	14.4	ND
R1 (acequinocyl-OH) [4]	9.3	ND
AKM-18 [4]	27.1	ND
Phthalic acid	NA	33.3-42.1
Total identified	**50.8**	**33.3-42.1**
Polar unknown(s)	4.1	50-54.5
Minor unknowns	0.7	3.2-10.0
Unresolved radioactivity	2.4	1.6-6.3
Residual solids (PES)	42.7	NA
Total recovery	100.7	99.5-100.9

ND = not detected; NA = not applicable.

1 The chemical names and structures for acequinocyl and its putative metabolites are presented in Table C.3.1.

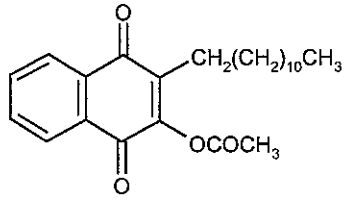
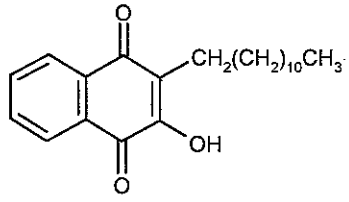
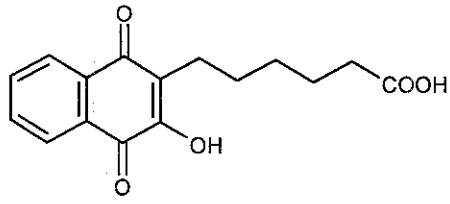
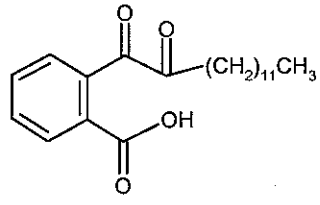
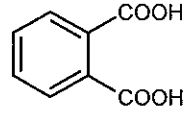
2 Data are from HPLC analysis of extracts from Pooled Fecal sample (Study Days 2-5) and are expressed as a percentage of % radioactivity in the analyzed sample. Feces from Days 2-5 accounted for 61.3% of the administered dose.

3 Data are from the separate 1D-TLC analyses of urine from Study Days 2-5; however, movement of radioactivity away from the origin was limited. HPLC analysis of Day 2 urine detected a peak of radioactivity at the R_f of phthalic acid.

4 Identities of parent, R1 and AKM-18 in fecal extracts were not confirmed.

C.3. Proposed Metabolic Profile

TABLE C.3.1. Identification of Compounds from Goat Metabolism Study. [1]

Common name/code	Chemical name	Chemical structure
Acequinocyl AKD-2023	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione	
Acequinocyl-OH (Metabolite R1)	2-dodecyl-3-hydroxy-1,4-naphthalenedione	
AKM-15	NA	
AKM-18	2-(1,2-dioxotetradecyl)-benzoic acid	
phthalic acid		

[1] Identification of parent and all metabolites are tentative.

D. CONCLUSION

The [^{14}C]acequinocyl goat metabolism study is adequate. Although the goat used in the study was dosed at the minimum level recommended by the Agency, the resulting levels of radioactivity in milk and tissues were too low to provided adequate identification of the ^{14}C-residues. TRR levels in tissues exceeded 0.05 ppm only in liver and kidneys, and characterization and identification of ^{14}C-residues in these tissues was limited. In addition, there is no reason that a higher dosing level could not have been used in the study, as evidenced by the cattle feeding study (45651610.der2) in which cows were dosed at levels equivalent to ~50 ppm in the diet.

Based on the data, the metabolism of acequinocyl in goats appears to involve loss of the acetyloxy moiety to form Metabolite R1 (also referred to as acequinocyl-OH) and partial cleavage of the dodecyl side chain to form AKM-15. Opening and degradation of the quinone ring was also evidenced by the presence of AKM-18 and phthalic acid.

E. REFERENCES

45651610.der2

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.2.1, 7.2.2, 7.2.3 and 7.4/OPPTS 860.1340/OECD IIA 4.2.5, 4.2.6 and 4.3
Residue Analytical Method - Apple and Orange

Primary Evaluator: Sarah Levy, Chemist
Registration Action Branch 1 (RAB1)
Health Effects Division (HED; 7509C)

Approved by: George F. Kramer, Ph.D., Chemist
RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 06-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651604 Carringer, S. (2001) Magnitude of the Residue of Acequinocyl and its Metabolite in Apple Raw Agricultural and Processed Commodities. Lab Project Number: TCI-00-001. Unpublished study prepared by Morse Laboratories, Inc. 397 p.

45651606 Carringer, S. (2002) Magnitude of the Residue of Acequinocyl and its Metabolite in Orange Raw Agricultural and Processed Commodities. Lab Project Number: TCI-01-003. Unpublished study prepared by Morse Laboratories, Inc. 329 p.

EXECUTIVE SUMMARY:

Method validation trials were conducted using a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse Method #Meth-133, Revision 3) for determining residues of acequinocyl and its metabolite, acequinocyl-OH, in/on apple and orange matrices. For this method, residues are extracted by homogenizing with acetonitrile (ACN) or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix, or with hexane (citrus oil and dehydrated pulp). Residues are then cleaned up by ACN:hexane partitioning, gel-permeation chromatography (GPC) (dehydrate pulp only), and using silica gel solid-phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on all matrices except citrus oil, which has a LOQ of 0.5 ppm for each analyte. The limit of detection (LOD) for all analytes in/on all matrices was not reported.

The HPLC/MS/MS Method (#Meth-133) is adequate for determining residues of acequinocyl and its metabolite, acequinocyl-OH, in/on apple and orange matrices. Recoveries from apple fruit, juice, and wet pomace samples fortified with each analyte at 0.01 or 0.5 ppm averaged 84-96% for acequinocyl and 76-81% for acequinocyl-OH. Recoveries from orange fruit, juice, and dried pulp fortified at 0.01 or 0.5 ppm averaged 80-101% for acequinocyl and 76-99% for

acequinocyl-OH, and recoveries from citrus oil at 0.5 or 25 ppm averaged 98 and 99% for acequinocyl and acequinocyl-OH, respectively.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the method validation data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations were noted that would impact the acceptability the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

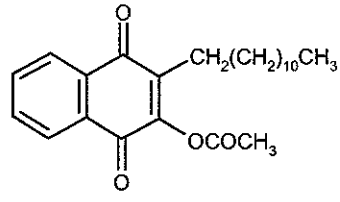
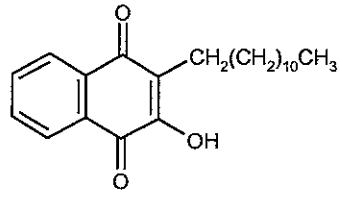
TABLE A.1. Nomenclature of Test Compound and Metabolite	
Compound	
Common name	Acequinocyl
Company experimental names	TM-413 or AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC
Compound	
Common name	Acequinocyl-OH
Company experimental names	R1, TM-413-OH, or OH-AKD-2023

TABLE A.1. Nomenclature of Test Compound and Metabolite	
IUPAC name	2-dodecyl-3-hydroxy-1,4-naphthoquinone
CAS name	2-dodecyl-3-hydroxy-1,4-naphthalenedione
CAS #	57960-31-3

TABLE A.2. Physicochemical Properties		
Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 acetone: >250 ethyl acetate: >250 xylene: >250 methanol: 6.1 n-heptane: 36 n-octanol: 29.2	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. MATERIALS AND METHODS

B.1. Data-Gathering Method (Morse Laboratories method, Meth-133, Revision #3, dated 4/02/01, with modifications dated 5/23/01 and 6/21/01)

B.1.1. Principle of the Method:

With the exceptions of citrus oil and dehydrated citrus pulp (described below), residues in/on fruit matrices are extracted by homogenizing with ACN or ACN:water. Aqueous samples (e.g. juices) are extracted with ACN only. Samples with high moisture contents (>80%) are extracted with ACN:water (10:1, v/v), and samples with lower moisture contents (50-80%) are extracted with ACN:water (5:4, v/v). The aqueous extract is separated into water and ACN layers by mixing with NaCl and centrifuging. The resulting ACN layer is dried by filtering through sodium sulfate, and residues are partitioned into hexane, dried through sodium sulfate and concentrated. For oily matrices, residues are back partitioned into ACN (hexane-saturated), concentrated to dryness, and redissolved in hexane. Residues in the concentrated hexane fractions from all matrices are then purified using a silica-gel SPE cartridge. Residues in hexane are loaded onto the cartridge, and the cartridge is washed with hexane and hexane:ethyl ether (49:1, v/v), discarding the eluates. Residues are then eluted with hexane:ethyl acetate (9:1, v/v), concentrated to dryness, and redissolved in ACN:acetone:0.4% aqueous formic acid (2:2:1, v/v) for HPLC analysis.

For citrus oil, residues are extracted directly with hexane, partitioned into ACN, concentrated to dryness, and redissolved in hexane. Residues are then cleaned up using a silcia gel SPE cartridge. Residues are loaded onto the cartridge, washed with hexane, eluted with hexane:ethyl acetate (9:1, v/v) and concentrated to dryness. Residues are then redissolved in ACN:acetone:0.4% aqueous formic acid (2:2:1, v/v) for HPLC analysis.

For dehydrated citrus pulp, residues are extracted directly with hexane, filtered, partitioned into ACN, concentrated to dryness, and redissolved in dichloromethane. Residues are first cleaned up using GPC. Residues are eluted through the GPC column with dichloromethane, concentrated to dryness and redissolved in hexane. Residue are then cleaned up using a silica gel SPE cartridge as describe above for citrus oil. Residues eluted from the SPE cartridge are concentrated to dryness and then redissolved in ACN:acetone:0.4% aqueous formic acid (2:2:1, v/v) for HPLC analysis.

The method notes that both analytes are very sensitive to light; therefore, amber/dark glassware should be used during analysis or glassware should be covered with aluminum foil. Rotary evaporator flasks should also be covered when concentrating samples.

Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a C_{18} column with a mobile phase gradient of water to methanol (each containing 0.1% formic acid) at a column temperature of 35°C. Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for acequinocyl and acequinocyl-OH is 0.01 ppm in/on all matrices, except citrus oil (LOQ = 0.5 ppm). The LOD for all analytes in/on all matrices was not reported.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Acequinocyl and Acequinocyl-OH Residues in Apple and Orange Fruit and Processed Commodities.

Method ID	Morse Laboratories Method #Meth-133, Revision #3
Analytes	Acequinocyl, Acequinocyl-OH
Extraction solvent/technique	ACN or ACN:water depending on matrix moisture content
Cleanup strategies	hexane:ACN partitioning, GPC column (dried pulp only), and silica SPE cartridges
Instrument/Detector	Reverse-phase (C_{18}) HPLC using a mobile phase gradient of water to methanol (both containing 0.1% formic acid) at a column temperature of 35° C. Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH.
Standardization method	External standards
Stability of std solutions	35 days at 1-8° C in dark
Retention times	Acequinocyl: ~9.2 min.; Acequinocyl-OH: ~10.5 min.

B.1.2. Method Validation

For method validation, 6-10 control samples of each apple and orange matrix were separately fortified with acequinocyl or acequinocyl-OH at 0.01 and 0.5 ppm, or 0.5 and 25 ppm (citrus oil). Fortified samples were analyzed for each compound along with control samples using the procedures described above.

The reports also included concurrent method recovery results from apple and orange field trials.

B.2. Enforcement Method

The proposed enforcement method is the same as the data-gathering method.

B.2.1. Independent Laboratory Validation (ILV)

An ILV trial of the proposed enforcement method has been successfully completed (MRID 45782301).

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method (Meth-133, Revision #3)

In the apple method validation trial, recoveries from apple fruit and processed commodities fortified with each analyte at 0.01 or 0.5 ppm were all within the acceptable 70-120% range for acequinocyl and acequinocyl-OH from all matrices, except one acequinocyl-OH recovery of 60% from whole fruit (Table C.1.1). Acequinocyl recoveries averaged 91% from fruit, 96% from juice, and 84% from wet pomace, with low standard deviations (± 5-8%). Acequinocyl-OH recoveries were slightly lower, but acceptable, averaging 78% from fruit, 81% from juice, and 76% from wet pomace, with low standard deviations (± 3-9%). Apparent residues of each analyte were <0.01 ppm in/on all control samples.

In the orange method validation trial, recoveries from orange fruit and processed commodities fortified with each analyte were all within the acceptable 70-120% range for acequinocyl and acequinocyl-OH from all matrices (Table C.1.1). Acequinocyl recoveries averaged 80% from fruit, 80% from juice, 101% from dried pulp, and 98% from oil, with low standard deviations (± 5-9%). Acequinocyl-OH recoveries averaged 76% from fruit, 81% from juice, 94% from dried pulp, and 99% from oil, with low standard deviations (± 5-13%). Apparent residues of each analyte were <LOQ (<0.01 or <0.5 ppm) in/on all control samples.

TABLE C.1.1. Recovery Results from Method Validation Trial for HPLC/MS/MS Method Meth-133 using Apple and Orange Matrices.

Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Apple						
Whole Fruit	0.01, 0.5	10	70-100	91 ± 8	60-90 (1)[1]	78 ± 9
Juice	0.01, 0.5	6	89-103	96 ± 6	78-86	81 ± 3
Wet Pomace	0.01, 0.5	6	78-90	84 ± 5	73-83	76 ± 4
Orange						
Whole Fruit	0.01, 0.5	10	73-89	80 ± 6	70-82	76 ± 5
Juice	0.01, 0.5	6	70-92	80 ± 9	70-89	81 ± 7
Dried Pulp	0.01, 0.5	6	91-109	101 ± 7	88-101	94 ± 5
Citrus Oil	0.5, 25.0	6	93-106	98 ± 5	84-116	99 ± 13

[1] The number of recoveries outside the 70-120% range is presented in parentheses.

In addition to the above method validation data, Arvesta provided concurrent recovery data from apple and orange field trials and processing studies, reviewed in separate DERs with this petition.

Concurrent method recoveries from apple fruit and processed commodities fortified with each analyte at 0.01-2.0 ppm were all within the acceptable 70-120% for acequinocyl and acequinocyl-OH from all matrices (Table C.1.2.1). Acequinocyl recoveries averaged 96% from fruit, 101% from juice, and 83% from wet pomace, with relatively low standard deviations (± 8 to 13%, n=2-6). Acequinocyl-OH recoveries were slightly lower, but acceptable, averaging 78% from fruit, 86% from juice, and 71% from wet pomace, with low standard deviations (± 2-5%, n=2-6). Apparent residues of each analyte were <0.01 ppm in/on all control samples.

TABLE C.1.2.1 Concurrent Recovery Results from Apple Field Trials Trial for HPLC/MS/MS Method Meth-133.[1]

Apple Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Whole Fruit	0.01-2.0	6	82-115	96 ± 13	70-83	78 ± 5
Juice	0.01, 0.5	2	92, 110	101	85, 87	86
Wet Pomace	0.01-2.0	3	76-91	83 ± 8	70-73	71 ± 2

[1] Concurrent recovery data are from submitted apple field trials and an apple processing study (MRID 45651604.der3, MRID 45651604.der4).

Concurrent method recoveries from orange fruit and processed commodities fortified with each analyte at 0.01-100 ppm were all within the acceptable 70-120% range for acequinocyl and acequinocyl-OH from all matrices (Table C.1.2.2). Acequinocyl recoveries averaged 86% from fruit and 101% from oil, and were 84% from one juice and 103% from one dried pulp sample. Acequinocyl-OH recoveries averaged 78% from fruit and 98% from oil, and were 86% from one

juice and 91% from one dried pulp sample. Apparent residues of each analyte were <LOQ (<0.01 or <0.5 ppm) in/on all control samples.

As the analytical method used the same solvent extraction as the plant metabolism studies, radiolabeled method validation data are not required. In addition, a confirmatory method is not required as the HPLC/MS/MS is sufficiently specific.

TABLE C.1.2.2 Concurrent Recovery Results from Orange Field Trials Trial for HPLC/MS/MS Method Meth-133. [1]

Orange Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Whole Fruit	0.01-1.0	7	72-100	86 ± 10	70-90	78 ± 8
Juice	0.01	1	84	NA	86	NA
Dried Pulp	0.5	1	103	NA	91	NA
Citrus Oil	0.5, 100	2	98, 104	101	87, 108	98

[1] Concurrent recovery data are from submitted orange field trials and an orange processing study (MRID 45651606.der2, MRID 45651606.der3).

NA = not applicable.

TABLE C.1.3. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Acequinocyl and Acequinocyl-OH Residues in Apple and Orange Fruit and Processed Commodities.

Analytes	Acequinocyl and Acequinocyl-OH
Equipment ID	A PE Sciex API 2000 HPLC/MS/MS with autosampler, an integrated Shimadzu chromatograph, a DGU-14A Degasser, and a *SCL*-10Avp System Controller
LOQ	Acequinocyl and Acequinocyl-OH: 0.01 ppm for each matrix except citrus oil (0.05 ppm)
LOD	not reported
Accuracy/Precision	Average method recoveries were 76-101% for acequinocyl and acequinocyl-OH from all matrices with standard deviations of ±3-13%.
Linearity	Example standard curves for acequinocyl and acequinocyl-OH at concentrations from 0.05-0.5 µg/mL had correlation coefficients of >0.999.
Specificity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

C.2. Enforcement Method

The enforcement method is the same as the data-gathering method.

C.3. ILV

An ILV was conducted according to guideline specifications (MRID 45782301). Method recoveries averaged 92 ± 4% for acequinocyl and 70 ± 4% for acequinocyl-OH. No method modifications were suggested by the independent laboratory.

D. CONCLUSION

The Morse Laboratories HPLC/MS/MS Method #Meth-133, Revision 3, was successfully validated using samples of apple fruit, wet pomace and juice, and orange fruit, juice, dried pulp, and oil. The validated LOQ for both acequinocyl and acequinocyl-OH is 0.01 ppm in each matrix except citrus oil (0.5 ppm).

E. REFERENCES

45782301 Faltynski, K. (2002) Independent Laboratory Validation (ILV) of Morse Laboratories' Analytical Method #METH-133, Revision #3, Entitled, "Determination of Acequinocyl and Acequinocyl-OH in Fruit Crops: Lab Project Number: 01-0036. Unpublished study prepared by EN-CAS Analytical Laboratories. 90 p.

45651604.der3
45651604.der4
45651606.der2
45651606.der3

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta Corporation
DACO 7.2.1, 7.2.2, 7.2.3 and 7.4/OPPTS 860.1340/OECD IIA 4.2.5, 4.2.6 and 4.3
Residue Analytical Method - Almonds

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 20-NOV-2002). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651609 Carringer, S. (2002) Magnitude of the Residue of Acequinocyl and its Metabolite in Almond Raw Agricultural Commodities. Lab Project Number: TCI-01-005. Unpublished study prepared by Morse Laboratories, Inc. 236 p.

EXECUTIVE SUMMARY:

A method validation trial was conducted using a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse Method #Meth-135) for determining residues of acequinocyl and its metabolite, acequinocyl-OH, in/on almond hulls and nutmeats. For this method, residues are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate. Residues are then purified by solvent partitioning, gel permeation chromatography (GPC), and silica gel solid-phase extraction (SPE). Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid) with either a C18 (parent) or phenyl-hexyl (metabolite) column. Residues are detected and quantified by MS/MS detection in the positive ion mode by monitoring the transition of m/z 385 to 189 for parent and the transition of m/z 343 to 189 for acequinocyl-OH. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on both nutmeats and hulls. The limit of detection (LOD) for all analytes in/on all matrices was not reported.

The HPLC/MS/MS Method (#Meth-135) is adequate for determining residues of acequinocyl and its metabolite, acequinocyl-OH, in/on almond hulls and nutmeats. Method validation recoveries from samples fortified with each analyte at 0.01 or 0.05 ppm averaged 90 ± 7% for acequinocyl and 92 ± 8% for acequinocyl-OH from almond nutmeats and 90 ± 12% for acequinocyl and 95 ± 5% for acequinocyl-OH from almond hulls.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the method validation data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations were noted that would impact the acceptability the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

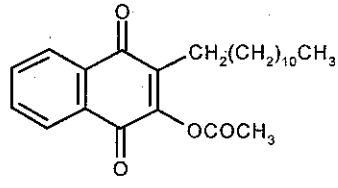
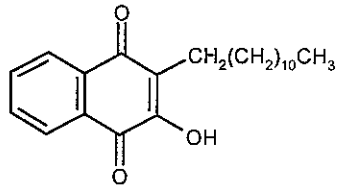
TABLE A.1. Nomenclature of Test Compound and Metabolites	
Compound	
Common name	Acequinocyl
Company experimental names	TM-413 or AKD-2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC
Compound	
Common name	Acequinocyl-OH
Company experimental names	R1, TM-413-hydroxy, or AKD-2023-OH
IUPAC name	2-dodecyl-3-hydroxy-1,4-napthoquinone
CAS name	2-dodecyl-3-hydroxy-1,4-naphthalenedione,
CAS #	57960-31-3

TABLE A.2. Physicochemical Properties		
Parameter	Value	Reference (MRID#)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (mg/L at 20°C)	1,2-dichloroethane: >250 ethyl acetate: >250 methanol: 6.1 n-octanol: 29.2 acetone: >250 xylene: >250 n-heptane: 36	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK$_a$)	no measurable pK$_a$	45434905
Octanol/water partition coefficient Log(K$_{OW}$)	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. MATERIALS AND METHODS

B.1. Data-Gathering Method (Morse Laboratories method, Meth-135, dated 10/19/01)

B.1.1. Principle of the Method (Morse Laboratories Method #Meth-135):

Residues in/on nutmeats and hulls are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate, filtered, and concentrated. Residues are then partitioned repeatedly with hexane:acetonitrile (1:3, v/v), discarding the hexane layer. Residues in the acetonitrile (ACN) layer are concentrated to dryness and redissolved in dichloromethane. Residues are then purified by GPC eluting with dichloromethane. Residues in the collected fraction are concentrated to dryness, redissolved in hexane and further purified by silica gel SPE. After loading residues, the cartridge is washed with hexane. Residues are eluted with hexane:ethyl acetate (9:1, v/v), concentrated to dryness, and redissolved in ACN:acetone:0.4% formic acid (2:2:1, v/v) for HPLC analysis.

The method noted that both analytes are very sensitive to light; therefore, amber/dark glassware should be used during analysis or glassware should be covered with aluminum foil. Rotary evaporator flasks should also be covered when concentrating samples.

Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). A C_{18} column is used for analysis of nutmeats, and a Phenyl-Hexyl column is used for analysis of hulls; both types of columns are maintained at 35°C during analysis. Residues are detected and quantified by MS/MS detection in the positive ion mode. For residues in almond nutmeats, the transition of

m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. For residues in almond hulls, the transition of m/z 343 to 189 was monitored for parent and acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on all matrices. The LOD for all analytes in/on all matrices was not reported.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Acequinocyl and Acequinocyl-OH Residues in Almond Nutmeats and Hulls.	
Method ID	Morse Laboratories Method #Meth-135
Analytes	Acequinocyl, Acequinocyl-OH
Extraction solvent/technique	Hexane in the presence of anhydrous sodium sulfate
Cleanup strategies	Hexane:acetonitrile (3:1, v/v) partitioning, gel permeation chromatography, and silica gel SPE cartridges
Instrument/Detector	Reverse-phase HPLC using a mobile phase gradient of water to methanol (both containing 0.1% formic acid) with either a C_{18} (parent) or phenyl-hexyl (metabolite) column at a temperature of 35°C. Residues are detected and quantified by MS/MS detection in the positive ion mode. For residues in almond nutmeats, the transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. For residues in almond hulls, the transition of m/z 343 to 189 was monitored for parent and acequinocyl-OH.
Standardization method	External standards
Stability of std solutions	35 days at 1-8°C
Retention times	Nutmeats: acequinocyl: ~ 18.9 min.; acequinocyl-OH: ~17.1 min. Hulls: acequinocyl: ~ 30.5 min.; acequinocyl-OH: ~27.6 min.

B.1.2. Method Validation

For method validation, 10 control samples of each almond matrix were separately fortified with acequinocyl or acequinocyl-OH at 0.01 or 0.5 ppm. Fortified samples were analyzed for each compound along with control samples using the procedures described above.

The report also included concurrent method recovery results from almond field trials.

B.2. Enforcement Method

The purposed enforcement method is the same as the data-gathering method.

B.2.1. Independent Laboratory Validation (ILV)

An ILV trial of the proposed enforcement method has been successfully completed (MRID 45651602).

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method (Meth-135)

Method validation recoveries from almond nutmeat and hull samples fortified separately with each analyte at 0.01 or 0.5 ppm were all within the acceptable 70-120% range, except from one nutmeat sample with unusually low recoveries for both analytes (49 and 52%), which were considered outliers and were not used to calculate means or SD (Table C.1.1). For samples fortified separately with each analyte at 0.01 or 0.5 ppm, method validation recoveries were 90 ± 7% for acequinocyl and 92 ± 8% for acequinocyl-OH from almond nutmeats and 90 ± 12% for acequinocyl and 95 ± 5% for acequinocyl-OH from almond hulls.

In the field trials, procedural recoveries were 94 ± 23% for acequinocyl and 68 ± 7% for acequinocyl-OH from 3 nutmeat control samples fortified separately with each analyte at 0.01-0.05 ppm. Procedural recoveries for hulls were 91 ± 16% for acequinocyl and 85 ± 13% for acequinocyl-OH from 5 control samples fortified separately with each analyte at 0.05-5.0 ppm.

TABLE C.1.1. Recovery Results from Method Validation Trial for HPLC/MS/MS Method Meth-135 using Almond Matrices.

Almond Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Hulls	0.01, 0.5	10	77-105	90 ± 7	78-107	92 ± 8
Nutmeats	0.01, 0.5	9	77-108 (52)[1]	90 ± 12	89-101 (49)[1]	95 ± 5

[1] The recoveries in parentheses represent outliers which were not included in the calculation of the mean and SD.

TABLE C.1.3. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Acequinocyl and Acequinocyl-OH Residues in Almond Nutmeats (ILV).

Analytes	Acequinocyl and Acequinocyl-OH
Equipment ID	A PE Sciex API 2000 LC/MS/MS with autosampler, an integrated Shimadzu chromatograph,, and a *SCL*-10Avp System Controller
LOQ	Acequinocyl and Acequinocyl-OH: 0.01 ppm
LOD	Not reported
Accuracy/Precision	Average method recoveries were 90 ± 7% from hulls and 90 ± 12 from nutmeats for acequinocyl, and 92 ± 8% from hulls and 95 ± 5% from nutmeats for acequinocyl-OH.
Linearity	Example standard curves for acequinocyl and acequinocyl-OH at concentrations from 0.025-0.25 µg/mL had correlation coefficients of >0.998.
Specificity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

C.2. Enforcement Method

The purposed enforcement method is the same as the data-gathering method.

C.3. ILV

An ILV was conducted according to guideline specifications (MRID 45651602). Method recoveries averaged 96 ± 6% for acequinocyl and 71 ± 4% for acequinocyl-OH. No method modifications were suggested by the independent laboratory.

D. CONCLUSION

The Morse Laboratories HPLC/MS/MS Method #Meth-135 was successfully validated using samples of almond hulls and nutmeats. The validated LOQ for both acequinocyl and acequinocyl-OH is 0.01 ppm in each matrix.

E. REFERENCES

45651602 Faltynski, K. (2002) Independent Laboratory Validation (ILV) of Morse Laboratories' Analytical Method #METH-135, Original, Entitled, "Determination of Acequinocyl and Acequinocyl-OH in Almond Nutmeats and Almond Hulls: Lab Project Number: 01-0044. Unpublished study prepared by EN-CAS Analytical Laboratories. 85 p.

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.2.1, 7.2.2, 7.2.3 and 7.4/OPPTS 860.1340/OECD IIA 4.2.5, 4.2.6 and 4.3
Residue Analytical Method - Livestock

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 10-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651610 Phillips, J. (2002) Magnitude of the Residue of TM-413 (Acequinocyl) and OH-TM-413 (OH-Acequinocyl) in Dairy Cow Milk and Tissues. Lab Project Number: ABC Study #47474. Unpublished study prepared by ABC Laboratories. 270 p.

EXECUTIVE SUMMARY:

A method validation trial was conducted on a high-performance liquid chromatography (HPLC)/ mass spectrometry (MS)/MS method (Morse Laboratories Method #Meth-139, revision #2) for determining residues of acequinocyl and its metabolite, acequinocyl-OH, in/on livestock commodities. For this method, residues are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate, purified by solvent partitioning, gel-permeation chromatography (GPC), and cleanup using silica gel solid-phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid) with a C_{18} column. Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on all livestock matrices. The limit of detection (LOD) for all analytes in/on all matrices was not reported.

The HPLC/MS/MS Method (Meth-139) is adequate for determining residues of acequinocyl and its metabolite, acequinocyl-OH, in milk and livestock commodities. For samples (n=10) fortified with acequinocyl at 0.01 or 0.1 ppm, recoveries averaged 102% from milk, 95% from muscle, 90% from liver, 107% from kidney, and 83% from fat. For samples (n=10) fortified with acequinocyl-OH at 0.01 or 0.1 ppm, recoveries averaged 93% from milk, 101% from muscle, 105% from liver, 100% from kidney, and 87% from fat.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the residue analytical method data are classified as scientifically acceptable. The reported recoveries of acequinocyl from liver and kidney samples were calculated from the sum of acequinocyl and acequinocyl-equivalents found as acequinocyl-OH. The calculations resulted in slightly higher reported recoveries for these matrices, but this did not affect the overall acceptability of the recoveries.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations were noted that would impact the acceptability the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

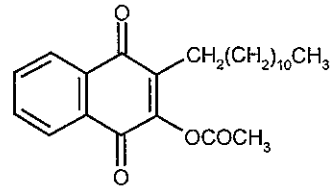
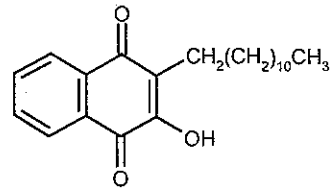
TABLE A.1. Nomenclature of Test Compound and Metabolite	
Compound	
Common name	Acequinocyl
Company experimental names	TM-413 and AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC
Compound	
Common name	Acequinocyl-OH
Company experimental names	*R1, TM-413 Hydroxy, or AKD-2023-OH*
IUPAC name	2-dodecyl-3-hydroxy-1,4-naphthoquinone
CAS name	2-dodecyl-3-hydroxy-1,4-naphthalenedione
CAS #	57960-31-3

TABLE A.2. Physicochemical Properties of Acequinocyl				
Parameter	Value			Reference (MRID)
Melting point/range	59.6 C			45434906
pH	6.94			45434904
Density	1.13 g/cm^3			45434904
Water solubility (20°C)	6.69 µg/L			45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 ethyl acetate: >250 methanol: 6.1 n-octanol: 29.2	acetone: >250 xylene: >250 n-heptane: 36		45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals			45434905
Dissociation constant (pK$_a$)	no measurable pK$_a$			45434905
Octanol/water partition coefficient Log(K$_{OW}$)	≥6.2			45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362			45434905

B. MATERIALS AND METHODS

B.1. Data-Gathering Method (Morse Laboratories Method #Meth-139, Revision #2)

B.1.1. Principle of the Method:

Residues from all samples are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate, and the resulting extracts are then filtered and concentrated. Residues are then partitioned repeatedly with hexane:acetonitrile (1:3, v/v), discarding the hexane layer. Residues in the acetonitrile (ACN) layer are concentrated to dryness and redissolved in dichloromethane. Residues are then purified by GPC eluting with dichloromethane. Residues in the collected fraction are concentrated to dryness, redissolved in hexane and further purified by silica gel SPE. After loading residues, the SPE cartridge is washed with hexane. Residues are eluted with hexane:ethyl acetate (9:1, v/v), concentrated to dryness, and redissolved in ACN:acetone:0.4% formic acid (2:2:1, v/v) for HPLC analysis.

The method noted that both analytes are very sensitive to light; therefore, amber/dark glassware should be used during analysis or glassware should be covered with aluminum foil. Rotary evaporator flasks should also be covered when concentrating samples.

Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid) with a C_{18} column maintained at 35°C during analysis. One gradient is used for the analysis of milk, muscle, and kidney and another gradient is used for analysis of liver and fat. Residues are detected and

quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on all matrices. The LOD for all analytes in/on all matrices was not reported.

TABLE B.1.1. Summary Parameters for the Analytical Method Used for the Quantitation of Acequinocyl and Acequinocyl-OH Residues in Dairy Cow Matrices.	
Method ID	Morse Laboratories Method #Meth-139, revision #2
Analytes	Acequinocyl, Acequinocyl-OH
Extraction solvent/technique	hexane in the presence of anhydrous sodium sulfate
Cleanup strategies	hexane:acetonitrile (3:1, v/v) partitioning, gel permeation chromatography, and solid phase extractions using silica gel SPE cartridges
Instrument/Detector	*Reverse-phase HPLC using a mobile phase gradient of water to methanol (both containing* 0.1% formic acid) with a C_{18} column maintained at 35°C. Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH.
Standardization method	External standards
Stability of std solutions	35 days at 1-8° C
Retention times	Milk, Muscle, and Kidney : acequinocyl: ~ 13.9 min.; acequinocyl-OH: ~12.6 min. *Liver and Fat: acequinocyl: ~ 25.4 min.; acequinocyl-OH: ~24.5 min.*

B.1.2. Method Validation

For method validation, 10 control samples of each cow matrix was fortified with parent acequinocyl and acequinocyl-OH at 0.01 and 0.1 ppm. Samples of liver and kidney were fortified separately with each analyte. Fortified samples were analyzed along with control samples using the procedures described above.

The report also included concurrent method recovery results from a cow feeding study.

B.2. Enforcement Method

The proposed enforcement method is the same as the data-gathering method.

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method (Meth-139)

In the method validation trial, recoveries from milk and cow tissues fortified with acequinocyl and acequinocyl-OH at 0.01 and 0.1 ppm were all within the acceptable 70-120% range for all matrices (Table C.1.1). Acequinocyl recoveries averaged 102% from milk, 95% from muscle, 90% from liver, 107% from kidney, and 83% from fat, with low standard deviations for all matrices (± 4-8%). The reported recoveries of acequinocyl from liver and kidney samples were calculated from the sum of acequinocyl and acequinocyl-equivalents found as acequinocyl-OH. The calculations resulted in slightly higher reported recoveries for these matrices, but this did not affect the overall acceptability of the recoveries. Acequinocyl-OH recoveries averaged 93% from milk, 101% from muscle, 105% from liver, 100% from kidney, and 87% from fat, with low standard deviations for all matrices (± 4-12%). Apparent residues of each analyte were <0.01 ppm in/on all control samples.

TABLE C.1.1. Recovery Results from Method Validation Trial of the HPLC/MS/MS Method Meth-139 using Dairy Cow Matrices.

Cow Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl (ACEQ)		Acequinocyl-OH (ACEQ-OH)	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Milk	0.01, 0.1	10	90-110	102 ± 7	76-102	93 ± 9
Muscle	0.01, 0.1	10	85-105	95 ± 6	87-113	101 ± 9
Liver	0.01, 0.1	10	76-101 [1]	90 ± 8	90-120	105 ± 12
Kidney	0.01, 0.1	10	98-119 [1]	107 ± 8	82-113	100 ± 9
Fat	0.01, 0.1	10	78-90	83 ± 4	82-95	87 ± 4

[1] The reported recoveries of ACEQ from liver and kidney samples were calculated from the sum of ACEQ and ACEQ-equivalents found as ACEQ-OH.

In addition to the above method validation data, Arvesta provided concurrent recovery data from the cow feeding study, reviewed in a separate DER with this petition.

Concurrent method recoveries from milk and cow tissues fortified with each analyte at 0.01-0.5 ppm were all within the acceptable 70-120% from all matrices (Table C.1.2). Acequinocyl recoveries averaged 99% from milk, 101% from muscle, 91% from liver, 94% from kidney, and 91% from fat. Acequinocyl-OH recoveries averaged 91% from milk, 83% from muscle, 75% from liver, 74% from kidney, and 90% from fat. Apparent residues of each analyte were <0.01 ppm in/on all control samples.

TABLE C.1.2 Concurrent Recovery Results from Dairy Cow Feeding Study of the HPLC/MS/MS Method Meth-139. [1]

Cow Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Milk	0.01-0.1	16	85-111	99 ± 8	72-110	91 ± 12
Muscle	0.01-0.1	3	96-105	101 ± 5	77-91	83 ± 7
Liver	0.05, 0.1	2	95, 86	91	72, 78	75
Kidney	0.1, 0.5	2	94, 94	94	70, 78	74
Fat	0.02, 0.5	2	90, 91	91	91, 89	90

[1] Concurrent recovery data are from livestock feeding study (45651610.der2).

The extractability of residues with Meth-139 was not assessed using samples from the goat metabolism study. In the goat metabolism study, ^{14}C-residues were extracted with ACN, ACN:water and methanol. A confirmatory method is not required as the HPLC/MS/MS is sufficiently specific.

TABLE C.1.3. Characteristics for the Data-Gathering Analytical Method Used for the Quantitation of Acequinocyl and Acequinocyl-OH Residues in Dairy Cow Matrices.

Analytes	Acequinocyl and Acequinocyl-OH
Equipment ID	A PE Sciex API 2000 high performance liquid chromatograph with autosampler, an integrated Shimadzu chromatograph, a DGU-14A Degasser, and a *SCL*-10Avp System Controller
LOQ	Acequinocyl and Acequinocyl-OH: each 0.01 ppm
LOD	not reported
Accuracy/Precision	All recoveries were in the acceptable 70-120% range. Average method recoveries for acequinocyl ranged from 83 ± 4% from fat to 107 ± 8% from kidney, and for acequinocyl-OH ranged from 87 ± 4% from fat to 105 ± 12% from liver.
Linearity	Example standard curves for acequinocyl and acequinocyl-OH at concentrations from 0.025-0.25 µg/mL had correlation coefficients of >0.999.
Specificity	The control chromatograms generally have no peaks above the chromatographic background and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

C.2. Enforcement Method

The enforcement method is the same as the data-gathering method.

D. CONCLUSION

The HPLC/MS/MS Method Morse Laboratories Method #Meth-139, revision #2, was successfully validated using samples of milk and dairy cow tissues. The validated LOQ is 0.01 ppm for both acequinocyl and acequinocyl-OH.

E. REFERENCES

45651610.der2

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.2.4/OPPTS 860.1360/OECD IIIA 5.3.1
Multiresidue Analytical Methods

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651603 Fomenko, J. (2001) Evaluation of TM-413 and Hydroxy-TM-413 Through the FDA Multiresidue Methods: Lab Project Number: A055.001. Unpublished study prepared by Maxim Technologies, Inc. 107 p.

EXECUTIVE SUMMARY:

Acequinocyl and its hydroxy metabolite (acequinocyl-OH) were tested using the Food and Drug Administration (FDA) Multiresidue Method Protocols. As acequinocyl and acequinocyl-OH are not N-methylcarbamate or substituted urea compounds, they were not tested through Protocols A or G. Acequinocyl was also not tested through Protocol B as it does not have an acid or phenol moiety. However, acequinocyl-OH was evaluated using Protocol B. Both parent and the hydroxy metabolite were evaluated through Protocols C-F.

Acequinocyl was adequately recovered using Method 302, E1 + C1 with gas chromatography (GC)/electron-capture detector (ECD); recoveries of parent from fortified apples (0.1 and 0.5 ppm) were 69-82%. However, acequinocyl can not be recovered using Method 303 or 304, due to poor recoveries from the Florisil cleanup.

Acequinocyl-OH could be completely recovery using Method 402, E1 or E2 + C1 with GC/ECD. Recoveries of acequinocyl-OH were 80.4-106% from fortified samples of apples (0.05 and 0.5 ppm) and 82.0-111% from fortified samples of almonds (0.05 and 0.5 ppm). However, the hydroxy metabolite cannot be recovered using Methods 302, 303, or 304.

The submitted data will be forwarded to the U.S. FDA for further evaluation.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the multiresidue method testing data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance and Data Confidentiality statements were provided.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

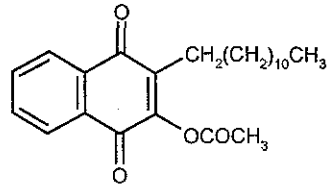
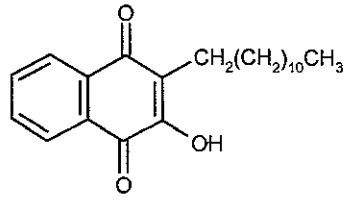
TABLE A.1. Nomenclature of Test Compound and Metabolite	
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	*3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate*
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC
Compound	
Common name	Acequinocyl-OH
Company experimental names	R1, TM-413 Hydroxy, AKD-2023-OH
IUPAC name	2-dodecyl-3-hydroxy-1,4-naphthoquinone
CAS name	2-dodecyl-3-hydroxy-1,4-naphthalenedione
CAS #	*57960-31-3*

TABLE A.2. Physicochemical Properties of Acequinocyl		
Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 ethyl acetate: >250 methanol: 6.1 n-octanol: 29.2 acetone: >250 xylene: >250 n-heptane: 36	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. MATERIALS AND METHODS

Acequinocyl, acequinocyl-OH and methylated acequinocyl-OH were evaluated using FDA Multiresidue Protocols. None of these compounds were tested using Protocol A as these compounds were not N-methylcarbamates, and acequinocyl was not evaluated through Protocol B as it has neither an acid or phenol structure. However, acequinocyl-OH was evaluated using Protocol B. Both acequinocyl and acequinocyl-OH were also evaluated using Protocols C-F. (Note: although not reported in the submission, none of the compounds were tested through Protocol G, as these compounds are not substituted urea compounds).

C. RESULTS AND DISCUSSION

The testing of acequinocyl and acequinocyl-OH through FDA Multiresidue Method Testing Protocols is summarized in Table C.1.

For Protocol C, acequinocyl, acequinocyl-OH, and methylated acequinocyl-OH each eluted from the DB-1, DB-17, and Rtx-225 columns under standard GC conditions (Table C.2), although the parent compound produced two responses in Modules DG-1 and DG-13. Each compound gave a good to excellent response on the ECD and no response on the FID. A DB-1 column with ECD was used for evaluations in Protocols B and D-F.

Acequinocyl was not tested through Protocol B as it does not contain an acid or phenol group. However, acequinocyl-OH, which has a phenol group, was evaluated through Protocol B. The hydroxy metabolite was methylated using TBAH/CH_3I as outlined in Section 402 C1b and its

recovery through the GPC and Florisil column cleanups was evaluated. Methylated acequinocyl was completely recovered through both the GPC and Florosil cleanup steps (Section 402 C1a). Therefore, the complete method was evaluated using duplicate fortified samples of apples (0.05 and 0.5 ppm) and almonds (0.05 and 0.5 ppm). The recovery of acequinocyl-OH through Method 402, E1 or E2 + C1 using GC/ECD was 80.4-106% from apples and 82.0-111% from almonds.

In Protocol D, the recovery of acequinocyl from the Florisil column cleanup was 83.8% using the C1 eluant and 77.1% using the C5 eluants. Therefore, acequinocyl was evaluated through the complete method using duplicate samples of apples fortified at 0.1 and 0.5 ppm. Recoveries of acequinocyl through Method 302 E1+ C1 were 68.9-83.2%, and recoveries through Method 302 E1 + C5 were 51.1-68.3%. As the hydroxy metabolite was not recovered from the Florisil cleanup step, its recovery through the complete method (Section 302) was not evaluated.

In both Protocols E and F, acequinocyl and acequinocyl-OH were not recovered (<30%) from the required Florisil cleanup procedures; therefore, the recovery of these analytes through the complete methods (Sections 303 and 304) were not evaluated.

TABLE C.1. Results of Multiresidue Methods Testing with Acequinocyl and Acequinocyl-OH.

PAM I Protocol	Results	Comments
A	Acequinocyl and acequinocyl-OH do not process an N-methylcarbamate structure and were therefore not evaluated through Protocol A.	
B	As acequinocyl is not an acid or phenol, it was not tested through Protocol B. Acequinocyl-OH was completely recovered from samples of apples and almonds each fortified in duplicate at 0.05 and 0.5 ppm. Recoveries through Method 402 E1 or E2 + C1 were 80-106% from apples and 82-111% from almonds.	Multiresidue Method 402 may be adequate for detecting residues of the hydroxy metabolite.
C	Acequinocyl, acequinocyl-OH, and methylated acequinocyl-OH each eluted from the DB-1, DB-17, and Rtx-225 columns under standard GC conditions. Each compound gave a good to excellent response on ECD and no response on FID.	GC/ECD analysis was used for testing through Protocols B, D -F.
D	Acequinocyl was recovered from samples of apples fortified in duplicate at 0.1 and 0.5 ppm. Recoveries through Method 302 E1+ C1 were 68.9-83.2%, and recoveries through Method 302 E1 + C5 were 51.1-68.3% Acequinocyl-OH was not recovered from the Florisil cleanup step.	Multiresidue Method 302, E1 + C1 may be adequate for detecting residues of the acequinocyl, but not the hydroxy metabolite
E	As neither acequinocyl nor acequinocyl-OH were recovered from Florisil using C1 or C2 eluants, the complete method was not evaluated.	
F	As neither acequinocyl nor acequinocyl-OH were recovered from Florisil using C1 or C2 eluants, the complete method was not evaluated.	
G	Acequinocyl and acequinocyl-OH are not substituted ureas; therefore, these compounds were not evaluated through Protocol G.	

Table C.2. GC Responses for Acequinocyl, Acequinocyl-OH, and Methylated Acequinocyl-OH.			
Module	**Column/Detector**	**RRT [1]**	**ng for 50% FSD**
Acequinocyl			
DG-1	DB-1/ECD	12.44, 17.39 [3]	100, 17
DG-10 [2]	DB-1/ECD	2.56	8.9
DG-13	DB-17/ECD	10.47, 16.77 [3]	43, 23
DG-18	Rtx-225/ECD	No response	--
DG-6	DB-1/FID	No response	--
Acequinocyl-OH			
DG-1	DB-1/ECD	12.32	20
DG-10 [2]	DB-1/ECD	1.95	12
DG-13	DB-17/ECD	10.23	18
DG-18	Rtx-225/ECD	No response	--
DG-6	DB-1/FID	No response	--
Methylated Acequinocyl-OH			
DG-1	DB-1/ECD	13.14	7.9
DG-10 [2]	DB-1/ECD	2.04	5.5
DG-13	DB-17/ECD	10.95	6.9
DG-18	Rtx-225/ECD	9.33	9.0
DG-6	DB-1/FID	No response	--

[1] Depending on module, the relative retention time (RRT) was compared to either DDT, permethrin, chloropyrifos, or ethion.
[2] Analysis was conducted at Level II temperature (225 C).
[3] Compound produced two responses on this system.

D. CONCLUSION

Acequinocyl could be adequately recovered (69-82%) from fortified samples of apples (0.1 and 0.5 ppm) using Method 302, E1 + C1 with GC/ECD, and its metabolite, acequinocyl-OH, could be completely recovered (80-111%) from fortified samples of apples and almonds using Method 402, E1 or E2 + C1 with GC/ECD. The hydroxy metabolite could not be recovered using Method 302, and neither compound could be recovered using Methods 303 or 304.

These data will be forwarded to forwarded to the U.S. FDA for further evaluation.

E. REFERENCES

None

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.3/OPPTS 860.1380/OECD IIA 6.1.1 and IIIA 8.1.1
Storage Stability - Apple Fruit and Processed Commodities

Primary Evaluator Sarah Levy, Chemist
Registration Action Branch 1 (RAB1)
Health Effects Division (HED; 7509C)

Approved by George F. Kramer, Ph.D., Chemist
RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 21-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651604 Carringer, S. (2001) Magnitude of the Residue of Acequinocyl and its Metabolite in Apple Raw Agricultural and Processed Commodities. Lab Project Number: TCI-00-001. Unpublished study prepared by Morse Laboratories, Inc. 397 p.

EXECUTIVE SUMMARY:

In a storage stability study, control samples of apple whole fruit (RAC), juice, and wet pomace were fortified with acequinocyl or its metabolite, acequinocyl-OH, each at 1.0 ppm and stored frozen for up to 5 months, with analyses at 0, 1, 3, and 5 months.

The high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse Meth-133) for determining residues of acequinocyl and acequinocyl-OH in/on apple matrices has been validated and was found to be adequate for data collection (45651604.der1).

The storage stability data are adequate and indicate that acequinocyl and acequinocyl-OH are stable in frozen (-20 C) apple fruit, juice, and wet pomace for up to 5 months.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the storage stability data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

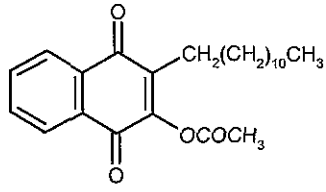
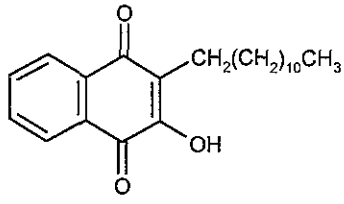
TABLE A.1. Nomenclature of Test Compound and Metabolite	
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC
Compound	
Common name	Acequinocyl-OH
Company experimental names	R1, TM-413 Hydroxy, AKD-2023-OH
IUPAC name	2-dodecyl-3-hydroxy-1,4-naphthoquinone
CAS name	2-dodecyl-3-hydroxy-1,4-naphthalenedione
CAS #	57960-31-3

TABLE A.2. Physicochemical Properties		
Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 ethyl acetate: >250 methanol: 6.1 n-octanol: 29.2 acetone: >250 xylene: >250 n-heptane: 36	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Sample Preparation

Control samples of apple RAC, juice, and wet pomace were fortified separately with each analyte at 1.0 ppm. Details of the fortification process were not provided. Two subsamples were then extracted and analyzed to establish day zero recoveries; the remaining samples were placed in freezer (-25 ± 5 C) storage for up to 5 months.

B.2. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-133) for determining residues of acequinocyl and acequinocyl-OH in/on apple matrices has been validated and was found to be adequate for data collection (45651604.der1). A brief description of the methods follows.

Residues are extracted by homogenizing with acetonitrile (ACN) or ACN:water (depending on matrix) and purified by solvent partitioning and by eluting residues through silica solid-phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid) with a C_{18} column Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on all matrices. The limit of detection (LOD) for all analytes in/on all matrices was not reported.

C. RESULTS AND DISCUSSION

Based on the results of the method validation trial (45651604.der1), the HPLC/MS/MS method (Morse Meth-133) is adequate for collecting data on acequinocyl and acequinocyl-OH residues in/on apple matrices.

Control apple fruit, juice, and wet pomace samples were fortified separately with acequinocyl or acequinocyl-OH at 1.0 ppm and stored frozen for up to 5 months, with analyses at 0, 1, 3, and 5 months. Residues of acequinocyl and acequinocyl-OH were stable in all apple matrices for up to 5 months (Tables C.1.1 and C.1.2).

TABLE C.1.1 Stability of Acequinocyl in Apple Matrices Following Storage at -20 ± 5°C.

Commodity	Spike level (mg/kg)	Storage interval (months)	Recovery (%)		
			Freshly Fortified	Stored Samples [1]	Corrected [1]
Apple Fruit (RAC)	1.0	0	91, 91	NA [2]	100
		1	79, 87	90, 96	109, 116
		3	105, 91	95, 102	97, 104
		5	83, 84	87, 104	104, 107
Apple Juice	1.0	0	81, 91	NA	100
		1	77, 81	89, 85	112, 108
		3	98, 113	95, 92	90, 87
		5	94, 94	94, 89	100, 94
Apple Wet Pomace	1.0	0	81, 83	NA	100
		1	71, 95	87, 105	105, 126
		3	95, 95	84, 104	88, 109
		5	94, 97	85, 87	88, 91

[1] Reported recoveries were corrected for procedural (freshly fortified) recoveries and control contribution.
[2] NA = not applicable. Two freshly fortified samples were analyzed at day zero.

TABLE C.1.2 Stability of Acequinocyl-OH in Apple Matrices Following Storage at -20 ± 5°C.

Commodity	Spike level (mg/kg)	Storage interval (months)	Recovery (%)		
			Freshly Fortified [3]	Stored Samples [3]	Corrected [1,3]
Apple Fruit (RAC)	1.0	0	87, 91	NA [2]	NA
		1	72, 81	66, 61	86, 80
		3	109, 95	90, 79	88, 77
		5	86, 89	88, 84	101, 93
Apple Juice	1.0	0	77, 84	NA	NA
		1	76, 82	76, 83	96, 105
		3	88, 101	89, 90	94, 95
		5	90, 90	89, 89	99, 99
Apple Wet Pomace	1.0	0	75, 73	NA	NA
		1	65, 84	76, 74	102, 99
		3	84, 88	86, 78	100, 90
		5	86, 87	74, 77	86, 88

1 Reported recoveries were corrected for procedural (freshly fortified) recoveries.
2 NA = not applicable. Two freshly fortified samples were analyzed at day zero.
3 Reported recoveries were corrected for control contributions.

D. CONCLUSION

The storage stability data are adequate and indicate that acequinocyl and acequinocyl-OH are stable in frozen apple matrices for up to 5 months. Samples from the apple field trials and processing study (MRID 45651604) were stored frozen for a maximum of 5 months.

E. REFERENCES

45651604.der1

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.3/OPPTS 860.1380/OECD IIA 6.1.1 and IIIA 8.1.1
Storage Stability - Orange Fruit and Processed Commodities

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 07-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651606 Carringer, S. (2002) Magnitude of the Residue of Acequinocyl and its Metabolite in Orange Raw Agricultural and Processed Commodities. Lab Project Number: TCI-01-003. Unpublished study prepared by Morse Laboratories, Inc. 329 p.

EXECUTIVE SUMMARY:

In a storage stability study, control samples of orange whole fruit (RAC), juice, dried pulp, and citrus oil were fortified separately with acequinocyl and acequinocyl-OH at 0.5 ppm or 25 ppm (oil only). Samples were stored frozen (-20 C) and analyzed at 5 months (RAC) or 3 months (juice, dried pulp, and oil).

The high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse Meth-133) for determining residues of acequinocyl and acequinocyl-OH in/on orange matrices has been validated and was found to be adequate for data collection (45651604.der1).

Based on analyses from the single storage interval (3 or 5 months), residues of acequinocyl and acequinocyl-OH appear to be somewhat stable in frozen orange matrices. After 5 months of frozen storage, acequinocyl was stable in whole oranges, but acequinocyl-OH had a decline of 23%. After 3 months of frozen storage, declines of both analytes were noted in juice (14-20%), dried pulp (27-32%), and oil (17-18%). Residue declines are comparable to declines of 3-9% per month. Samples from the orange field trials and processing study (DER 45651606.der2 and 45651606.der3) were stored frozen for a maximum of 3.7 months.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the storage stability data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP) quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

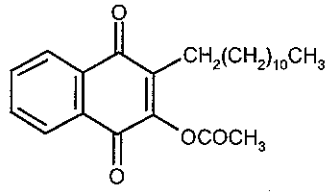
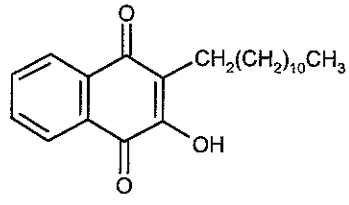
TABLE A.1. Nomenclature of Test Compound and Metabolites	
Compound	
Common name	Acequinocyl
Company experimental names	TM-413
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC
Compound	
Common name	Acequinocyl-OH
Company experimental names	TM-413 Hydroxy
IUPAC name	2-dodecyl-3-hydroxy-1,4-napthoquinone
CAS name	2-dodecyl-3-hydroxy-1,4-naphthalenedione
CAS #	57960-31-3

TABLE A.2. Physicochemical Properties		
Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 ethyl acetate: >250 methanol: 6.1 n-octanol: 29.2 acetone: >250 xylene: >250 n-heptane: 36	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Sample Preparation

Control samples of orange fruits, juice, dried pulp, and citrus oil were fortified separately with each analyte at 0.5 ppm or 25 ppm (oil only). Details of the fortification process were not provided. Samples were placed in freezer (-25 ± 5 C) storage for up to 5 months (whole fruit) or 3 months (juice, dried pulp, and oil).

B.2. Analytical Methodology

The HPLC/MS/MS method (Morse Meth-133, Revision #3) for determining residues of acequinocyl and acequinocyl-OH in/on orange matrices has been validated and was found to be adequate for data collection (45651604.der1). A brief description of the methods follows.

Residues are extracted by homogenizing with hexane (oil and dehydrated pulp) or with acetonitrile (ACN) or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning, gel-permeation chromatography (GPC) (dehydrate pulp only), and using silica-gel solid-phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on all matrices except citrus oil, which has a LOQ of 0.5 ppm for each analyte. The limit of detection (LOD) for all analytes in/on all matrices was not reported.

C. RESULTS AND DISCUSSION

Based on the results of the method validation trial (45651604.der1), the HPLC/MS/MS method (Morse Meth-133, Revision #3) is adequate for collecting data on acequinocyl and acequinocyl-OH residues in/on orange matrices.

Control orange fruit, juice, dried pulp, and citrus oil samples were fortified separately with acequinocyl and acequinocyl-OH at 0.5 ppm or 25 ppm (oil only). Samples were stored frozen and analyzed at 5 months (RAC) or 3 months (juice, dried pulp, and oil). Zero-day recoveries reported in the stability study were from samples used for method validation as the method validation and stability studies were conducted concurrently.

After 5 months of frozen storage, acequinocyl was stable in whole oranges, but acequinocyl-OH had a decline of 23%. After 3 months of frozen storage, declines of both analytes were noted in juice (14-20%), dried pulp (27-32%), and oil (17-18%). Residue declines were comparable to 3-9% per month.

TABLE C.1 Stability of Acequinocyl and Acequinocyl-OH in Orange Matrices Following Storage at -20 ± 5°C.

Orange Commodity	Spike level (mg/kg)	Storage interval (months)	Recovery (%)		
			Freshly Fortified (Mean)	Stored Samples	Corrected (Mean) [1]
Acequinocyl					
Fruit (RAC)	0.5	0 [2]	76-89 (**83**)	NA	100
		5	79, 71 (75)	86, 89	114, 119 (117)
Juice	0.5	0 [2]	85-92 (**88**)	NA	100
		3	90, 100 (95)	78, 73	82, 77 (80)
Dried Pulp	0.5	0 [2]	101-109 (**106**)	NA	100
		3	82, 61 (72)	49, 48	68, 67 (68)
Oil	25	0 [2]	99-106 (**102**)	NA	100
		3	128, 124 (126)	96, 112	76, 89 (83)
Acequinocyl-OH					
Fruit (RAC)	0.5	0 [2]	72-82 (**78**)	NA	100
		5	74, 71 (73)	50, 60	70, 83 (77)
Juice	0.5	0 [2]	82-89 (**86**)	NA	100
		3	76, 92 (84)	71, 73	85, 87 (86)
Dried Pulp	0.5	0 [2]	92-101 (**96**)	NA	100
		3	81, 61 (71)	51, 52	72, 74 (73)
Oil	25	0 [2]	103-116 (**109**)	NA	100
		3	121, 117 (119)	90, 104	75, 88 (82)

[1] Reported recoveries from stored samples were corrected for average procedural (freshly fortified) recoveries and control contribution. Day-0 recoveries from freshly fortified samples were corrected to 100%.

[2] Zero-day recoveries (average in **bold**) reported in the stability study were from samples used for method validation as the method validation and stability studies were conducted concurrently.

NA = not applicable

D. CONCLUSION

The storage stability data are adequate. Samples from the orange field trials and processing study (DERs 45651606.der2 and 45651606.der3) were stored frozen for a maximum of 3.7 months.

E. REFERENCES

45651604.der1
45651606.der2
45651606.der3

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.3/OPPTS 860.1380/OECD IIA 6.1.1 and IIIA 8.1.1
Storage Stability - Almonds

Primary Evaluator Sarah Levy, Chemist
Registration Action Branch 1 (RAB1)
Health Effects Division (HED; 7509C)

Approved by George F. Kramer, Ph.D., Chemist
RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 07-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651609 Carringer, S. (2002) Magnitude of the Residue of Acequinocyl and its Metabolite in Almond Raw Agricultural Commodities. Lab Project Number: TCI-01-005. Unpublished study prepared by Morse Laboratories, Inc. 236 p.

EXECUTIVE SUMMARY:

In a storage stability study, control samples of almond nutmeats and hulls were fortified with acequinocyl or its metabolite, acequinocyl-OH, each at 1.0 ppm. Samples were stored frozen (-20 C) and analyzed at 3.5 months.

The high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse Meth-135) for determining residues of acequinocyl and acequinocyl-OH in/on almond matrices has been validated and was found to be adequate for data collection (45651609.der1).

Based on analyses from the single storage interval (~3.5 months), residues of acequinocyl and acequinocyl-OH appear to be somewhat stable in frozen almond matrices. After ~3.5 months of frozen storage, acequinocyl residues declined 20% in almond nutmeats and 14% in almond hulls, and acequinocyl-OH residues declined 24% in almond nutmeats and 20% in almond hulls. Residue declines were comparable to 4-7% per month.

Samples from the almond field trials (DER 45651609.der3) were stored frozen for a maximum of 3.5 months.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the storage stability data are classified as scientifically acceptable.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

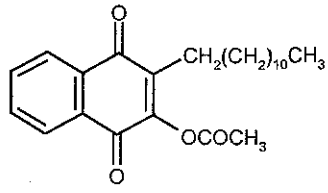
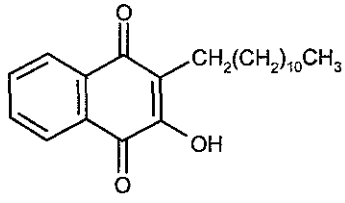
TABLE A.1. Nomenclature of Test Compound and Metabolite	
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC
Compound	
Common name	Acequinocyl-OH
Company experimental names	R1, TM-413 Hydroxy, AKD-2023-OH
IUPAC name	2-dodecyl-3-hydroxy-1,4-naphthoquinone
CAS name	2-dodecyl-3-hydroxy-1,4-naphthalenedione
CAS #	57960-31-3

TABLE A.2. Physicochemical Properties		
Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 ethyl acetate: >250 methanol: 6.1 n-octanol: 29.2 acetone: >250 xylene: >250 n-heptane: 36	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Sample Preparation

Control samples of almond nutmeats and hulls were fortified separately with each analyte at 0.5 ppm. Details of the fortification process were not provided. Samples were placed in freezer (-20 ± 5 C) storage for up to 104 days (3.5 months).

B.2. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-135) for determining residues of acequinocyl and acequinocyl-OH in/on almond matrices has been validated and was found to be adequate for data collection (45651609.der1). A brief description of the methods follows.

Residues are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate. Residues are then purified by solvent partitioning, gel-permeation chromatography (GPC), and silica gel solid-phase extraction (SPE). Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid) with either a C18 (parent) or phenyl-hexyl (metabolite) column. Residues are detected and quantified by MS/MS detection in the positive ion mode by monitoring the transition of m/z 385 to 189 for parent and the transition of m/z 343 to 189 for acequinocyl-OH. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on all matrices. The limit of detection (LOD) for all analytes in/on all matrices was not reported.

C. RESULTS AND DISCUSSION

Based on the results of the method validation trial (45651609.der1), the HPLC/MS/MS method (Morse Meth-135) is adequate for collecting data on acequinocyl and acequinocyl-OH residues in/on almond matrices.

Control almond nutmeat and hull samples were fortified separately with acequinocyl or acequinocyl-OH at 0.5 ppm. Samples were stored frozen and analyzed at 103-104 days (3.5 months). Zero-day recoveries reported in the stability study were from samples used for method validation as the method validation and stability studies were conducted concurrently.

After ~3.5 months of frozen storage, acequinocyl residues declined 20% in almond nutmeats and 14% in almond hulls, and acequinocyl-OH residues declined 24% in almond nutmeats and 20% in almond hulls. Residue declines were comparable to 4-7% per month.

TABLE C.1 Stability of Acequinocyl and Acequinocyl-OH in Almond Matrices Following Storage at -20 ± 5°C.

Commodity	Spike level (mg/kg)	Storage interval (days)	Recovery (%)		
			Freshly Fortified	Stored Samples [1]	Corrected [1]
Acequinocyl					
Almond Nutmeat	0.5	0 [2]	94-108 (**102**) [3]	NA	100
		103	110, 95 (102)	86, 78	84, 76 (80)
Almond Hulls	0.5	0 [2]	87-105 (**94**)	NA	100
		104	103, 94 (98)	88, 82	89, 83 (86)
Acequinocyl-OH					
Almond Nutmeat	0.5	0 [2]	93-101 (**98**) [3]	NA	100
		103	87, 83 (85)	67, 61	79, 72 (76)
Almond Hulls	0.5	0 [2]	86-107 (**91**)	NA	100
		104	86, 83 (85)	70, 64	83, 76 (80)

[1] Reported recoveries from stored samples were corrected for average procedural (freshly fortified) recoveries and control contribution. Day-0 recoveries from freshly fortified samples were corrected to 100%.

[2] Zero-day recoveries (average in **bold**) reported in the stability study were from samples used for method validation as the method validation and stability studies were conducted concurrently.

[3] Recoveries of each analyte from one 0-day nutmeat sample were unusually low (49 and 52%) and considered outliers which were not included in the calculation of the mean.

NA = not applicable

D. CONCLUSION

Samples from the almond field trials (DER 45651609.der3) were stored frozen for a maximum of 3.5 months.

E. REFERENCES

45651609.der1
45651609.der3

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.3/OPPTS 860.1380/OECD IIA 6.1.1 and IIIA 8.1.1
Storage Stability -Livestock

Primary Evaluator Sarah Levy, Chemist
Registration Action Branch 1 (RAB1)
Health Effects Division (HED; 7509C)

Approved by George F. Kramer, Ph.D., Chemist
RAB1/HED(7509C)

STUDY REPORTS:

MRID No. 45782302. Howell, R. (2002) Stability of TM-413 (Acequinocyl) and OH-TM-413 (OH-Acequinocyl) in Dairy Cow Milk and Tissues: Lab Project Number: 47380. Unpublished study prepared by ABC Laboratories. 145 pages.

EXECUTIVE SUMMARY:

Samples of milk, liver, kidney, muscle, and fat were spiked with acequinocyl, or its metabolite, acequinocyl-OH, each at a level of 0.5 ppm. The petitioner proposed that the mean (n=5) of method verification analyses performed in MRID 45651610 (fortified at 0.1 ppm) serve as zero day analyses. Samples were stored at -20 C $\pm$ 5C for a duration of 6-9 months. Due to the inadequacy of the study, it could not be determined if residues of parent or acequinocyl-OH increased or decreased in livestock tissue. The submitted storage stability data are inadequate (see below).

The high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse Meth-139, revision #2) for determining residues of acequinocyl and acequinocyl-OH in/on livestock matrices has been validated and was found to be adequate for data collection (45651610.der1).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the storage stability data are classified as scientifically unacceptable. As Day-0 samples were not analyzed, a determination of residue levels present at the time samples were placed in frozen storage could not be made. An insufficient number of time points were analyzed in order to establish that residues of acequinocyl or acequinocyl-OH were stable throughout duration of the study or to show how much of the residue was lost at various time points. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

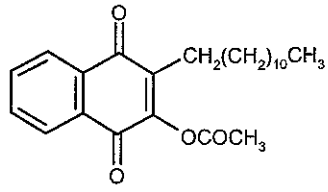
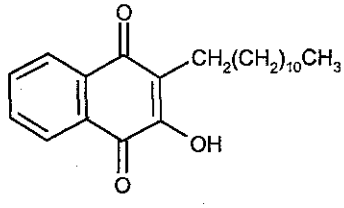
TABLE A.1. Test Compound Nomenclature	
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC
Compound	
Common name	Acequinocyl-OH
Company experimental names	R1, TM-413 Hydroxy, AKD-2023-OH
IUPAC name	2-dodecyl-3-hydroxy-1,4-naphthoquinone
CAS name	2-dodecyl-3-hydroxy-1,4-naphthalenedione
CAS #	57960-31-3

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound			
Parameter	Value		Reference
Melting point/range	59.6 C		45434906
pH	6.94		45434904
Density	1.13 g/cm^3		45434904
Water solubility (20°C)	6.69 µg/L		45434906
Solvent solubility (mg/L at 20°C)	1,2-dichloroethane: >250 ethyl acetate: >250 methanol: 6.1 n-octanol: 29.2	acetone: >250 xylene: >250 n-heptane: 36	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals		45434905
Dissociation constant (pK_a)	no measurable pK_a		45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2		45434906
UV/visible absorption spectrum	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362		45434905

B. EXPERIMENTAL DESIGN

B.1. Sample Handling and Preparation

Frozen stability was determined for acequinocyl and OH-acequinocyl in bovine milk, muscle, liver, kidney, and fat for the longest period as that for incurred-residue samples from MRID 45651610 under the same storage conditions (-20 C ± 5C). Stability samples were prepared by fortifying the control sample at 0.5 ppm, separately, with acequinocyl or OH-acequinocyl. All stability samples were fortified at method validation of the corresponding matrix. Samples intended for method controls and method fortifications were aliquoted and stored with the corresponding stability samples. Samples were stored frozen at (-20 C ± 5C) until the designated analysis period.

B.2. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-139, revision #2) for determining residues of acequinocyl and acequinocyl-OH in/on livestock matrices has been validated and was found to be adequate for data collection (45651610.der1). A brief description of the methods follows.

Residues are extracted with hexane in the presence of anhydrous sodium sulfate and concentrated for further partitioning with hexane/acetonitrile (ACN). Sample extracts were purified using gel-permeation chromatography (GPC), followed by silica-gel solid-phase extraction (SPE) cartridge clean-up. The purified extract was concentrated then analyzed by HPLC/MS/MS. Sample aliquot and final volume differences from the original method were required to analyze fat. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on all matrices, except fat. The LOQ in fat for both analytes was 0.02 ppm. The limit of detection (LOD) for all analytes in/on all matrices was not reported.

C. RESULTS AND DISCUSSION

Based on the results of the method validation trial (45651610.der1), the HPLC/MS/MS method (Morse Meth-139, revision #2) is adequate for collecting data on acequinocyl and acequinocyl-OH residues in/on livestock matrices

Samples of milk, liver, kidney, muscle, and fat were spiked with acequinocyl, or its metabolite, acequinocyl-OH, each at a level of 0.5 ppm. The mean (n=5) of method verification analyses performed in MRID 45651610 (fortified at 0.1 ppm) served as zero day analyses. Samples were stored at -20 C ± 5C for a duration of 6-9 months. Under these conditions, residues of acequinocyl and acequinocyl-OH appeared to decrease by <30% in milk, liver, kidney, muscle, and fat (Tables C.1.1 and C.1.2).

TABLE C.1. Summary of Concurrent Recoveries of Acequinocyl and Acequinocyl-OH in Livestock Tissues.					
Matrix	Spike level (mg/kg)	Storage Interval (days)	Sample size (n)	Recoveries (%)	Mean ± std dev
Acequinocyl					
Milk	0.50	287	1	116	NA
Muscle	0.50	229	2	112, 113	112.5 ± 0.7
Liver	0.50	246	2	113, 101	107 ± 8
Kidney	0.50	300	2	112, 127	119.5 ± 10
Fat	0.50	249	2	91.9, 119	105.5 ± 19
Acequinocyl-OH					
Milk	0.50	287	1	88.8	NA
Muscle	0.50	229	2	96.8, 99.2	98 ± 1.7
Liver	0.50	246	2	114, 98.4	106.2 ± 11
Kidney	0.50	300	2	119, 102	110.5 ± 12
Fat	0.50	249	2	109, 115	112 ± 4.2

TABLE C.2. Stability of Acequinocyl and Acequinocyl-OH] Residues in Livestock Tissues Following Storage at -20 C ± 5°C.				
Commodity	Spike level (mg/kg)	Storage interval (days)	% Recovery	Corrected % recovery*
Acequinocyl				
Milk	0.50	287	105, 95	90, 82
Muscle	0.50	229	87, 113	77, 101
Liver	0.50	246	80, 89	75, 83
Kidney	0.50	300	78	65
Fat	0.50	249	105, 103	99, 98
Acequinocyl-OH				
Milk	0.50	287	85, 91	95, 102
Muscle	0.50	229	84, 82	86, 83
Liver	0.50	246	89, 94	84, 88
Kidney	0.50	300	102, 97	92, 88
Fat	0.50	249	101, 105	90, 94

* Corrected for concurrent-recoveries

D. CONCLUSION

The submitted storage stability data are inadequate. As Day-0 samples were not analyzed, a determination of residue levels present at the time samples were placed in frozen storage could not be made. An insufficient number of time points were analyzed in order to establish that residues of acequinocyl or acequinocyl-OH were stable throughout duration of the study or to show how much of the residue was lost at various time points.

E. REFERENCES

45651610.der1

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 07-APR-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORTS:

45651610 Phillips, J. (2002) Magnitude of the Residue of TM-413 (Acequinocyl) and OH-TM-413 (OH-Acequinocyl) in Dairy Cow Milk and Tissues. Lab Project Number: ABC Study #47474. Unpublished study prepared by ABC Laboratories. 270 p.

EXECUTIVE SUMMARY:

In a ruminant feeding study, three groups of lactating dairy cows (4/group) were dosed orally twice a day for 28 consecutive days with gelatin capsules containing acequinocyl at target doses equivalent to 5, 15, and 50 ppm (5 DG, 15 DG, and 50 DG) in the feed on a dry weight basis. Based on the average daily dietary intake, the actual dose levels were equivalent to 4.9, 14.9, and 48.6 ppm of acequinocyl in the diet. Cows were milked twice daily, and composited daily milk samples from Study Days 1, 4, 8, 12, 16, 20, 24, and 28 were collected for analysis. Cows were sacrificed within 24 hours of the final dose, and samples of muscle, liver, kidney and fat samples were collected and stored frozen until analysis. The total frozen (ca. -20 C) storage intervals were 119-242 days for whole milk and 168-252 days for all tissues. Storage stability data and data pertaining to the stability of acequinocyl and acequinocyl-OH residues in frozen milk and tissues were not provided.

Milk and tissue samples were analyzed for acequinocyl and acequinocyl-OH (converted to acequinocyl-equivalents) residues using an adequate high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Meth-139). Briefly, residues are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate, purified by partitioning and gel-permeation chromatography (GPC) and by eluting residues through silica solid phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC and quantified by MS/MS detection in the positive ion mode. The limit of quantitation (LOQ) for combined acequinocyl residues is 0.02 ppm in milk and all tissues. The limits of detection (LODs) for both analytes were not reported. However, the LODs for each analyte have been estimated to be 0.001 ppm in milk, 0.002 ppm in muscle and fat, and 0.005 ppm in liver and kidneys.

With the exception of two fat samples, residues of acequinocyl were <LOD in all samples of

milk and tissues from all three dose groups. Acequinocyl residues above the LOD were found in samples of milk and tissues from each dose group, with the exceptions of kidney and muscle samples from the low dose group, which had residues <LOD.

In milk, the combined residues of acequinocyl and acequinocyl-OH (expressed in parent equivalents) were <0.002-0.003 ppm for the 5 ppm DG, <0.001-<0.005 ppm for the 15 ppm DG, and <0.003-0.013 ppm for the 50 ppm DG. Residues appeared to plateau by Study Day 4. Combined residues were <LOQ in all milk samples.

In liver, combined residues were <0.014-<0.032 ppm for the 5 ppm DG, <0.022-<0.037 ppm for the 15 ppm DG, and <0.059-<0.089 ppm for the 50 ppm DG. In kidneys, combined residues were <0.01 ppm for the 5 ppm DG, <0.010-<0.014 ppm for the 15 ppm DG, and <0.019-<0.040 ppm for the 50 ppm DG. In muscle, combined residues were <0.004 ppm for the 5 ppm DG, <0.004-<0.006 ppm for the 15 ppm DG, and <0.007-<0.008 ppm for the 50 ppm DG. In fat, combined residues in fat were <0.006-<0.013 ppm for the 5 ppm DG, <0.018-<0.030 ppm for the 15 ppm DG, and <0.044-<0.113 ppm for the 50 ppm DG.

For the 5, 15 and 50 DGs, the average combined residues were respectively <0.002, <0.003, and <0.006 ppm in milk; <0.023, <0.030, and <0.070 ppm in liver; <0.010, <0.012, and <0.034 ppm in kidneys; <0.004, <0.005, and <0.007 ppm in muscle; and <0.009, <0.022, and <0.075 ppm fat. The combined acequinocyl residues increased linearly with increased feeding level in liver and fat, but there was no linear relationship between feeding level and residues in the other tissues and milk.

Considering only the LOQ (0.01 ppm) for the two analytes, the combined residues were <LOQ in milk and muscle samples from all three dose groups and in kidney samples from the 5 and 15 ppm DG. Residues above the LOQ were found in liver and fat samples from each dose group and in kidney from the 50 ppm DG.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Provided that data supporting the storage stability of residues in livestock commodities are available, the feeding study data are classified as scientifically acceptable. To upgrade the feeding study, data are required depicting the frozen storage stability of acequinocyl and acequinocyl-OH in livestock milk and tissues.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations were noted that would impact the acceptability the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl. These uses could result in the transfer of residues to livestock by the feeding of wet apple pomace, dried citrus pulp, and/or almond hulls.

TABLE A.1.Test Compound Nomenclature

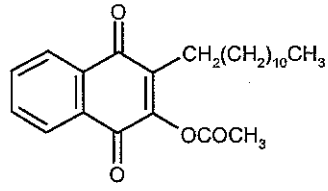
Compound	Chemical Structure
	
Common name	Acequinocyl
Company experimental name	TM-413 or AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use product/EP	1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties

Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20˚C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20˚C)	1,2-dichloroethane: >250 acetone: >250 ethyl acetate: >250 xylene: >250 methanol: 6.1 n-heptane: 36 n-octanol: 29.2	45434904
Vapor pressure at 25˚C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK$_a$)	no measurable pK$_a$	45434905
Octanol/water partition coefficient Log(K$_{OW}$)	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

The in-life phase of the feeding study was conducted by ABC Laboratories (Columbia, MO) from 4/16/01 to 5/22/01. Three groups of lactating dairy cows (4/group) were dosed orally once a day each morning for 28 consecutive days with gelatin capsules containing acequinocyl (97.1% purity) at levels equivalent to target concentrations of 5, 15, and 50 ppm in the feed on a dry weight basis. Based on the average daily dietary intake, the actual dose levels were equivalent to 4.9, 14.9, and 48.6 ppm of acequinocyl in the diet.

Cows were milked twice daily and subsamples of milk from Study Days 1, 4, 8, 12, 16, 20, 24, and 28 were collected for analysis. For each milk sample, subsamples from the p.m. milking were refrigerated overnight and pooled with the following morning's sample to form a single sample for a study day. The pooled sample was then stored at ca. -20 C. Within 24 hours of the final dose, cows were sacrificed and samples of liver, kidneys, muscle, and fat were collected. For each animal, equal amounts of loin and thigh muscle were composited for the muscle sample, and equal amounts of perirenal and omental fat were composited for the fat sample. Samples were coarsely chopped and placed in storage at ca. -20 C, until overnight shipment on dry ice to the analytical laboratory (Morse Laboratories, Sacramento, CA).

B.1. Livestock

TABLE B.1.1. Description of Livestock Used in the Feeding Study.

Species	Breed	Age	Weight at study initiation (kg)	Health status	Description of housing/holding area
Dairy Cow (*Bos taurus*)	Holstein	NS	516-770	Cows were free from any injury or illness that would preclude use in the study.	Housed individually in stanchions cushioned with rubber mats and cedar chip bedding, with feedbunks and automatic water cups

TABLE B.1.2. Dietary Regime.

Treatment group	Diet [1]	Water	Acclimation period	Predosing
All groups	*ad libitum* (twice daily)	*ad libitum*	6-7 days	None

[1] Feed was mixed in the following proportions: corn silage (46%), haylage (17%), chopped alfalfa hay (10%), and corn gluten feed, soybean meal and hulls, corn, chopped grass hay, fish meal, and fat (each ≤ 5%).

TABLE B.1.3. Dosing Regime.[1]

Treatment group	Treatment Type	Administered dose (mg/cow/day)	Food consumption (kg/cow/day)[2]	Residue intake in diet (ppm)[3]	Vehicle	Timing/ Duration
I	Oral[4]	102.2-118.0 (113)	21.1-26.0 (23.2)	4.3-5.5 (4.9)	gel capsule	daily/28 days
II		332.6-345.9 (338)	20.3-26.1 (22.8)	12.7-16.6 (14.9)		
III		958.2-1156.6 (1092)	18.1-26.8 (22.9)	39.0-62.3 (48.6)		

1 The range of values for administered dose, food consumption and residue in diet reflect the weekly averages for the individual cows over the 4-week study period. Averages for each treatment group over the entire study period are listed in parentheses.
2 Food consumption is expressed in a dry weight basis.
3 Target doses were 5, 15, and 50 ppm for treatment groups I, II, and III, respectively.
4 Capsules were administered in the morning using a balling gun.

TABLE B.1.4 Sample Collection.

Treatment group	Milk collected[1]	Average milk production (kg/cow/day)	Urine, feces and cage wash	Interval from last dose to sacrifice (days)	Tissues harvested and analysed[2]
I	twice daily	25.5	Not collected	within 24 hours	liver, kidney, muscle, and fat
II		23.9			
III		24.8			

1 Sub-samples of p.m. and a.m. milkings collected on study days 1, 4, 8, 12, 16, 20, 24, 28 were pooled by day.
2 Samples were collected from cows dosed daily for 28 days and sacrificed within 24 hours of the last dose.

B.2. Analytical Methodology (Morse Method #Meth-139)

The HPLC/MS/MS method (Morse Method #Meth-139, Revision 2) for determining residues of acequinocyl and acequinocyl-OH in livestock commodities was validated and found to be adequate for data collection (45651610.der1). A brief description of the method follows.

Residues from all samples are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate, and the resulting extracts are then filtered and concentrated. Residues are then partitioned repeatedly with hexane:ACN (1:3, v/v), discarding the hexane layer. Residues in the ACN layer are concentrated to dryness and redissolved in dichloromethane. Residues are then purified using GPC and silica gel SPE. The eluate is concentrated and residues are redissolved in ACN:acetone:0.4% formic acid (2:2:1, v/v) and filtered prior to HPLC analysis.

Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is each 0.01 ppm in/on all matrices. The LODs for both analytes were not reported. However, the LODs for each analyte have been estimated to be 0.001 ppm in milk, 0.002 ppm in muscle and fat, and 0.005 ppm in liver and kidneys.

C. RESULTS AND DISCUSSION

After collection, samples were stored (-20 C) at ABC until overnight shipment on dry ice to the analytical laboratory (Morse Laboratories, Sacramento, CA), where samples were stored at -20 C until analysis. Prior to analysis, the total frozen (ca. -20 C) storage intervals were 119-242 days for whole milk and 168-252 days for all tissues (Table C.2). Storage stability data were not provided and data pertaining to the stability of acequinocyl and acequinocyl-OH residues in frozen milk and tissues remains outstanding.

Based on the concurrent method recoveries, the HPLC/MS/MS method (#Meth-139) used in the feeding study is adequate for collecting data on acequinocyl and acequinocyl-OH in milk and tissues. Concurrent method recoveries from milk and cow tissues fortified with each analyte at 0.01-0.5 ppm were all within the acceptable 70-120% from all matrices (Table C.1). Average acequinocyl recoveries were 99% in milk, 101% in muscle, 91% in liver, 94% in kidney, and 91% in fat. Acequinocyl-OH recoveries averaged 91% from milk, 83% from muscle, 75% from liver, 74% from kidney, and 90% from fat. The method LOQ for each analyte in all matrices is 0.01 ppm; the LOQ for combined residues is 0.02 ppm. Apparent residues of each analyte were <0.01 ppm (<LOQ) in/on all control samples. Although the Method LODs were not reported, the reviewers estimated the LOD for each analyte to be 0.001 ppm for milk, 0.002 ppm for muscle and fat, and 0.005 ppm for liver and kidneys. Adequate sample calculations and chromatograms were provided.

Three groups of lactating dairy cows (4/group) were dosed orally once a day for 28 consecutive days with gelatin capsules containing acequinocyl at levels equivalent to 4.9, 14.9, and 48.6 ppm of acequinocyl in the diet. Cows were milked twice daily, and composited daily samples from Study Days 1, 4, 8, 12, 16, 20, 24, and 28 were collected for analysis. Cows were sacrificed within 24 hours of the final dose, and samples of muscle (thigh+loin), liver, kidney and fat (perirenal+omental) were collected and stored at -20 C until analysis.

In milk, residues of acequinocyl were below the LOQ (<0.01 ppm) in all 96 samples from all three dose groups (Table C.3.1) and were also below the LOD (<0.01 ppm) in all milk samples with the exception of two samples from the 5 ppm DG (0.002 ppm) and two samples from the 50 ppm DG (0.004 and 0.005 ppm). Residues of acequinocyl-OH were also below the LOQ (<0.01 ppm) in all milk samples from each dose group; however, acequinocyl residues were detectable (LOD, 0.001 ppm) in milk samples from each dose group. Residues of acequinocyl-OH (expressed in parent equivalents) in milk were <0.001-0.002 ppm for the 5 ppm DG, <0.001-0.004 ppm for the 15 ppm DG, and <0.001-0.008 ppm for the 50 ppm DG. Combined residues in milk were <0.002-0.003 ppm for the 5 ppm DG, <0.001-<0.005 ppm for the 15 ppm DG, and <0.003-0.013 ppm for the 50 ppm DG. Residues appeared to plateau by Study Day 4. Although below the LOQ, there was a tend toward increased residues with increased dose level.

In liver, residues of acequinocyl were <LOD (<0.005 ppm) in all four samples from each dose group; whereas, residues of acequinocyl-OH were >LOQ (>0.01 ppm) in all liver samples except

D. CONCLUSION

The study indicates that there is the potential for transfer of acequinocyl residues to livestock, most notably to liver and fat. Provided that data supporting the storage stability of residues in livestock commodities are available, the feeding study data are classified as scientifically acceptable. To upgrade the feeding study, data are required depicting the frozen storage stability of acequinocyl and acequinocyl-OH in livestock milk and tissues.

E. REFERENCES

45651610.der1

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Almonds

Primary Evaluator: Sarah Levy, Chemist
Registration Action Branch 1 (RAB1)
Health Effects Division (HED; 7509C)

Approved by: George F. Kramer, Ph.D., Chemist
RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 11-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45651609 Carringer, S. (2002) Magnitude of the Residue of Acequinocyl and its Metabolite in Almond Raw Agricultural Commodities. Lab Project Number: TCI-01-005. Unpublished study prepared by Morse Laboratories, Inc. 236 p.

EXECUTIVE SUMMARY:

In a total of 5 field trials conducted in CA during 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications at a 21-day retreatment interval (RTI) to almond trees during nut sizing to hull split at 0.297-0.303 lb ai/A/application, for a total of 0.597-0.603 lb ai/A/season. The number of crop field trials and geographic representation of the residue data on almond are adequate. Duplicate samples of almond nutmeats and hulls were collected at 7 days after the last application in four trials and at 0, 7, 21, and 35 days post-treatment in the residue decline trial.

Almond nutmeat and hull samples were stored frozen for a maximum of 90 days (nutmeat) or 104 days (hulls), prior to analysis. The submitted stability data for acequinocyl and acequinocyl-OH in almond nutmeats and hulls stored frozen for up to 104 days are adequate (45651609.der2). Therefore, the storage intervals from the field trials are supported by the available storage stability data.

Residues of acequinocyl and acequinocyl-OH in/on almond matrices were determined by a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse Meth-135) which was validated and found to be adequate for data collection (45651609.der1). For this method, residues are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate. Residues are then purified by solvent partitioning, gel-permeation chromatography (GPC), and silica-gel solid-phase extraction (SPE). Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC and quantified by MS/MS detection. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on both matrices. The limit of detection (LOD) was not reported.

Following two late-season foliar applications of the acequinocyl (1.25 lb/gal FlC) totaling 0.597-0.603 lb ai/A, combined residues of acequinocyl and acequinocyl-OH were <0.02 ppm (<LOQ) in/on all 10 almond nutmeat samples harvested 7 days post-treatment. Combined acequinocyl residues were also <0.02 ppm (<LOQ) in/on all 10 almond nutmeat samples harvested 0, 7, 21, and 35 days post-treatment. Combined acequinocyl residues were 0.432-1.28 ppm in/on 10 almond hull samples harvested 7 days post-treatment. Average combined acequinocyl residues in/on almond hull samples declined from 1.29 ppm at 0 days post-treatment to 0.296 ppm at 35 days post-treatment.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The almond field trial residue data are classified as scientifically acceptable under the conditions and parameters used in the study. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

TABLE A.1. Nomenclature of Test Compound and Metabolite

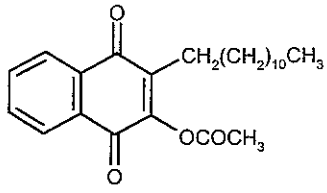
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties

Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 ethyl acetate: >250 methanol: 6.1 n-octanol: 29.2 acetone: >250 xylene: >250 n-heptane: 36	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Temperatures and rainfall data were collected at each site, and were within average historical values for the residue study period. Rainfall was supplemented with irrigation as needed.

TABLE B.1.1 Soil Characterization.

Study Location (City, State), Year	Soil Type
Hanford, CA, 2001	Clay Loam
Porterville, CA, 2001	Loam
Wasco, CA, 2001	Clay Loam
McFarland, CA, 2001	Sandy Loam
Terra Bella, CA, 2001	Sandy Loam

TABLE B.1.2. Study Use Pattern on Almond.

Location (City, State), Year	Application								Tank Mix Adjuvants
	Timing [1]	Formulation	Single Rate (lb a.i./A)	RTI [2] (days)	No. of Appl.	Method [3]	Volume (gal/A)	Total Rate (lb a.i./A)	
Hanford, CA, 2001	postemergence	1.25 lb/gal FIC	0.299	21	2	broadcast foliar	66	0.598	None
Porterville, CA, 2001	postemergence	1.25 lb/gal FIC	0.297-0.300	21	2	broadcast foliar	57-59	0.597	None
Wasco, CA, 2001	postemergence	1.25 lb/gal FIC	0.299-0.300	21	2	broadcast foliar	68	0.599	None
McFarland, CA, 2001	postemergence	1.25 lb/gal FIC	0.300-0.303	21	2	broadcast foliar	219-227	0.603	None
Terra Bella, CA, 2001	postemergence	1.25 lb/gal FIC	0.299-0.300	21	2	broadcast foliar	194-202	0.599	None

[1] All applications were made late in the season to mature, nut producing. Growth stage at application was from nut sizing to hull split..

[2] RTI = Retreatment Interval

[3] All applications were made using ground equipment.

TABLE B.1.3. Trial Numbers and Geographical Locations			
NAFTA Growing Region[1]	Total Almond Trials		
	Submitted	Requested	
		Canada	US
1	--	NA	--
2	--	NA	--
3	--	NA	--
4	--	NA	--
5	--	NA	--
6	--	NA	--
7	--	NA	--
8	--	NA	--
9	--	NA	--
10	5	NA	5
11	--	NA	--
12	--	NA	--
Total	5	NA	5

[1] Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the proposed use is for the US only.
NA = not applicable.

B.2. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-135) for determining residues of acequinocyl and acequinocyl-OH in/on almond matrices was validated and found to be adequate for data collection (45651609.der1). A brief description of the methods follows.

Residues are extracted by homogenizing with hexane in the presence of anhydrous sodium sulfate. Residues are then purified by solvent partitioning, GPC, and silica gel SPE. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid) with either a C18 (parent) or phenyl-hexyl (metabolite) column. Residues are detected and quantified by MS/MS detection in the positive ion mode by monitoring the transition of m/z 385 to 189 for parent and the transition of m/z 343 to 189 for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on both matrices. The LOD was not reported.

C. RESULTS AND DISCUSSION

The number of crop field trials and geographic representation of the residue data on almonds is adequate according to the latest EPA Guidance.

Duplicate samples of nutmeats and hulls were collected from each trial at each interval and placed in frozen storage within 3.5 hours of sampling. Samples were stored (-10 C) at the field sites for 5-29 days prior to shipment by freezer truck to the analytical laboratory (Morse Laboratories, Sacramento, CA), where samples were stored at -20 C until analysis. The total frozen (-20 ± 5 C) storage intervals were 61-90 days for almond nutmeat samples and 59-104 days for almond hulls samples (Table C.2). The submitted stability data for acequinocyl and acequinocyl-OH residues in/on almonds and hulls samples stored frozen for up to 104 days are adequate (45651609.der2). Therefore, the storage intervals from the field trials are supported by the available storage stability data.

The HPLC/MS/MS method (Morse #Meth-135) for determining residues of acequinocyl and acequinocyl-OH in/on almond matrices was validated and found to be adequate for data collection (45651609.der1). In the method validation trial, the average method recovery of acequinocyl was 90 ± 12% from almond nutmeats and 90 ± 7% from hulls, and the average method recovery of acequinocyl-OH was 95 ± 5% from almond nutmeats and 92 ± 8% from hulls. In the field trial analyses, concurrent method recoveries were 94 ± 23% for acequinocyl and 68 ± 7% for acequinocyl-OH from 3 nutmeat control samples fortified separately with each analyte at 0.01-0.05 ppm (Table C.1). Concurrent method recoveries for hulls were 91 ± 16% for acequinocyl and 85 ± 13% for acequinocyl-OH from 5 control samples fortified separately with each analyte at 0.05-5.0 ppm. Apparent residues of acequinocyl and acequinocyl-OH were each <0.01 ppm in/on all control almond nutmeat and hull samples. The LOQ for both analytes is 0.01 ppm in/on both matrices. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

In a total of 5 field trials conducted in CA during 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications at a 21-day RTI to almond trees during nut sizing and hull split at 0.297-0.303 lb ai/A/application, for a total of 0.597-0.603 lb ai/A/season (Table B.1.2.). Duplicate samples of almond nutmeats and hulls were collected at 7 days after the last application in four trials and at 0, 7, 21, and 35 days post-treatment in the residue decline trial.

Combined residues of acequinocyl and acequinocyl-OH were <0.02 ppm (<LOQ) in/on all 10 almond nutmeat samples harvested 7 days post-treatment. Combined acequinocyl residues were also <0.02 ppm (<LOQ) in/on all 10 almond nutmeat samples harvested 0, 7, 21, and 35 days post-treatment, therefore residue decline in nutmeats could not be calculated. Combined acequinocyl residues were 0.432-1.28 ppm in/on 10 almond hull samples harvested 7 days post-treatment (Table C.3). Average combined acequinocyl residues in/on almond hull samples declined from 1.29 ppm at 0 days post-treatment to 0.296 ppm at 35 days post-treatment.

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.1. Concurrent Recovery Results from Almond Field Trials Trial for HPLC/MS/MS Method Meth-135.

Almond Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Nutmeats	0.01-0.05	3	75-120	94 ± 23	60-74 (1)[1]	68 ± 7
Hulls	0.05-5.0	5	66-108 (1)	91 ± 16	71-102	85 ± 13

[1] The number of recoveries outside the acceptable 70-120% range is in parentheses.

TABLE C.2. Summary of Freezer Storage Conditions

Almond Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days)[1]
Nutmeats	-20 ± 5	61-90	103
Hulls		59-104	104

[1] The submitted stability data for acequinocyl and acequinocyl-OH in almond nutmeat and hull samples stored frozen for up to 104 days are inadequate (45651609.der2).

TABLE C.3. Almond Hulls Residue Data from Almond Field Trials with Acequinocyl.

Location (City, State), Year	EPA Region	Variety	Total Rate (lbs ai/A)	PHI (days)	Residues (ppm)[1]		
					Acequinocyl	Acequinocyl-OH	Combined[2]
Hanford, CA, 2001	10	Fritz	0.598	0	1.50, 0.968	0.075, 0.045	1.58, 1.01
				7	0.448, 0.436	0.038, 0.041	0.486, 0.477
				21	0.200, 0.302	0.026, 0.019	0.226, 0.321
				35	0.322, 0.236	0.019, 0.015	0.341, 0.251
Porterville, CA, 2001	10	Mission	0.597	7	0.632, 0.528	0.035, 0.029	0.667, 0.557
Wasco, CA, 2001	10	Nonpareil	0.599	7	0.636, 0.398	0.049, 0.034	0.685, 0.432
McFarland, CA, 2001	10	Mission	0.603	7	0.620, 0.665	0.039, 0.036	0.659, 0.701
Terra Bella, CA, 2001	10	Nonpareil	0.599	7	0.944, 1.22	0.048, 0.060	0.992, 1.28

[1] The LOQ for each analyte is 0.01 ppm.

TABLE C.4. Summary of Residue Data for Almond Nutmeats and Hulls from Almond Crop Field Trials with Acequinocyl.

Almond Matrix	Total Rate (lb a.i./A)	PHI (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [1]	Mean	Std. Dev.
Acequinocyl Residues								
Nutmeats	0.597-0.603	7 [2]	10	<0.01 [3]	<0.01	<0.01	0.01	NA
Hulls	0.597-0.603	7	10	0.398	1.22	1.08	0.653	0.253
Acequinocyl-OH Residues								
Nutmeats	0.597-0.603	7	10	<0.01 [3]	<0.01	<0.01	0.01	NA
Hulls	0.597-0.603	7	10	0.029	0.060	0.054	0.041	0.009
Combined Residues [4]								
Nutmeats	0.597-0.603	7	10	<0.02	<0.02	<0.02	<0.02	NA
Hulls	0.597-0.603	7	10	0.432	1.28	1.14	0.693	0.260

1 HAFT = Highest Average Field Trial.
2 The proposed PHI is 7 days.
3 The LOQ for both analytes is 0.01 ppm in/on almond nutmeats and hulls.
4 For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

D. CONCLUSION

The almond field trial residue data are adequate and reflect a maximum application rate of 0.603 lb ai/A (FlC) and a 7-day PHI.

E. REFERENCES

45651609.der1
45651609.der2

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Orange

Primary Evaluator: Sarah Levy, Chemist
Registration Action Branch 1 (RAB1)
Health Effects Division (HED; 7509C)

Approved by: George F. Kramer, Ph.D., Chemist
RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 19-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45651606 Carringer, S. (2002) Magnitude of the Residue of Acequinocyl and its Metabolite in Orange Raw Agricultural and Processed Commodities. Lab Project Number: TCI-01-003. Unpublished study prepared by Morse Laboratories, Inc. 329 p.

EXECUTIVE SUMMARY:

In a total of 12 field trials conducted in the U.S. during 2000 and 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications at a 21-day retreatment interval (RTI) to orange trees during the later stages of fruit development at 0.30-0.31 lb ai/A/application, for a total of 0.60-0.62 lb ai/A/season. The number of crop field trials and geographic representation of the residue data on orange are adequate. Duplicate orange fruit samples were collected at 7 days after the last application in eleven trials and at 0, 7, 21, 35, and 49 days post-treatment in the residue decline trial.

Orange fruit samples were stored frozen for a maximum of 110 days prior to analysis. The submitted stability data for acequinocyl and acequinocyl-OH in orange matrices stored frozen for up to 5 months (~150 days) are adequate (45651606.der1). Therefore, the storage intervals from the orange field trials are supported by the available storage stability data.

Residues of acequinocyl and acequinocyl-OH in/on orange matrices were determined by a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse #Meth-133, Revision 3), which was validated and found to be adequate for data collection (45651604.der1). For this method, residues are extracted by homogenizing with acetonitrile (ACN) or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica-gel solid-phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC and quantified by MS/MS detection in the positive ion mode. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on orange fruit. The limit of detection (LOD) was not reported.

Following two applications of the 1.25 lb/gal FlC totaling 0.60-0.62 lb ai/A, combined residues of acequinocyl and acequinocyl-OH were 0.018-0.290 ppm in/on 24 orange fruit samples harvested 7 days post-treatment. Average combined acequinocyl residues in/on orange fruit samples declined from 0.105 ppm at 0 days post-treatment to 0.013 ppm at 49 days post-treatment.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The orange field trial residue data are classified as scientifically acceptable under the conditions and parameters used in the study. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

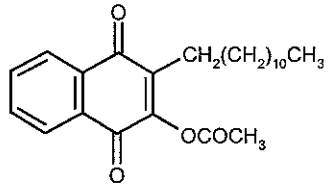
TABLE A.1. Nomenclature of Test Compound and Metabolite	
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties		
Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 acetone: >250 ethyl acetate: >250 xylene: >250 methanol: 6.1 n-heptane: 36 n-octanol: 29.2	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK$_a$)	no measurable pK$_a$	45434905
Octanol/water partition coefficient Log(K$_{OW}$)	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Temperatures and rainfall data were collected at each site, and were within average historical values for the residue study period. Rainfall was supplemented with irrigation as needed.

TABLE B.1.1 Soil Characterization.

Study Location (City, State), Year	Soil Type
Clermont, FL, 2001	Sand
Immokalee, FL, 2001	Fine Sand
DeLeon Springs, FL, 2001	Fine Sand
Clermont, FL, 2001	Sand
Oviedo, FL, 2000	Sand
Mims, FL, 2000	Sand
Winter Garden, FL, 2001	Fine Sand
St. Cloud, FL, 2001	Fine Sand
Raymondville, TX, 2001	Fine Sandy Loam
Porterville, CA, 2001	Loam
Richgrove, CA, 2001	Sandy Loam
Exeter, CA, 2001	Loam

TABLE B.1.2. Study Use Pattern on Orange.

Location (City, State), Year	Application								Tank Mix Adjuvants
	Timing [1]	Formulation	Single Rate (lb a.i./A)	RTI [2] (days)	No. of Appl.	Method [3]	Volume (gal/A)	Total Rate (lb a.i./A)	
Clermont, FL, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	168-171	0.60	None
Immokalee, FL, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	65	0.60	None
DeLeon Springs, FL, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	109-112	0.60	None
Clermont, FL, 2001	postemergence	1.25 lb/gal FlC	0.30	22	2	broadcast foliar	71	0.60	None
Oviedo, FL, 2000	postemergence	1.25 lb/gal FlC	0.31	21	2	broadcast foliar	71-72	0.62	None
Mims, FL, 2000	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	71	0.60	None
Winter Garden, FL, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	122-123	0.60	None
St. Cloud, FL, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	123-125	0.60	None
Raymondville, TX, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	249-252	0.60	None
Porterville, CA, 2001	postemergence	1.25 lb/gal FlC	0.30 1.18	21	2	broadcast foliar	53	0.60 2.36	None
Richgrove, CA, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	57-58	0.60	None
Exeter, CA, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	200-202	0.60	None

[1] The first application to trees was made during the immature to mature stage of fruit development and the second application was made when fruit were mature.
[2] RTI = retreatment interval
[3] All applications were made using ground equipment.

TABLE B.1.3. Trial Numbers and Geographical Locations

NAFTA Growing Region[1]	Total Orange Trials		
	Submitted	Requested[2]	
		Canada	US
1	--	NA	--
2	--	NA	--
3	8	NA	8
4	--	NA	--
5	--	NA	--
6	1	NA	1
7	--	NA	--
8	--	NA	--
9	--	NA	--
10	3	NA	3
11	--	NA	--
12	--	NA	--
Total	12	NA	12

[1] Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the proposed use is for the US only.
[2] The number of requested orange field trials when the proposed tolerance is for the citrus fruits crop group.
NA = not applicable.

B.2. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on orange fruit was validated and found to be adequate for data collection (45651604.der1). A brief description of the method follows.

Residues are extracted by homogenizing with ACN or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica-gel SPE cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on orange fruit. The LOD was not reported.

C. RESULTS AND DISCUSSION

The number of crop field trials and geographic representation of the residue data on oranges is adequate according to the latest EPA Guidance.

Duplicate samples of whole fruit were collected from each trial at each interval and placed in frozen storage within 3 hours of sampling. Samples were stored at the field sites for 0-47 days prior to shipment by freezer truck to the analytical laboratory (Morse Laboratories, Sacramento, CA), where samples were stored at -20 C until analysis. The total frozen (-20 ± 5 C) storage intervals were 15-110 days for fruit samples (Table C.2). The submitted stability data for acequinocyl and acequinocyl-OH residues in/on orange matrices stored frozen for 5 months (~150 days) are adequate (45651606.der1). Therefore, the storage intervals from the orange field trials are supported by the available storage stability data.

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on orange matrices was validated and found to be adequate for data collection (45651604.der1). Method recoveries from orange fruit, juice, dry pulp, and citrus oil samples fortified with each analyte at 0.01- 25 ppm averaged 80-101% for acequinocyl and 70-116% for acequinocyl-OH. In the field trial analysis, concurrent method recoveries were 86 ± 10% for acequinocyl and 78 ± 8% for acequinocyl-OH from seven orange fruit control samples fortified separately with each analyte at 0.01-1.0 ppm (Table C.1). Apparent residues of acequinocyl and acequinocyl-OH were <0.01 ppm in/on all control orange fruit samples. The LOQ for both analytes is 0.01 ppm in/on orange fruit. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

In a total of 12 field trials conducted in the U.S. during 2000 and 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to orange trees during the later stages of fruit development at 0.30-0.31 lb ai/A/application, for a total of 0.60-0.62 lb ai/A/season (Table B.1.2.). Duplicate orange fruit samples were collected at 7 days after the last application in eleven trials and at 0, 7, 21, 35, and 49 days post-treatment in the residue decline trial.

Combined residues of acequinocyl and acequinocyl-OH were 0.018-0.290 ppm in/on 24 orange fruit samples harvested 7 days post-treatment (Table C.3). Average combined acequinocyl residues in/on orange fruit samples declined from 0.105 ppm at 0 days post-treatment to 0.013 ppm at 49 days post-treatment.

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.1 Concurrent Recovery Results from Orange Field Trials Trial for HPLC/MS/MS Method Meth-133.

Orange Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Whole Fruit	0.01-1.0	7	72-100	86 ± 10	70-90	78 ± 8

TABLE C.2. Summary of Freezer Storage Conditions

Orange Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days) [1]
Fruit	-20 ± 5	15-110	~150 days

[1] The submitted stability data for acequinocyl and acequinocyl-OH in orange matrices stored frozen for up to 5 months (~150 days) are adequate (45651606.der1).

TABLE C.3. Orange Fruit Residue Data from Orange Field Trials with Acequinocyl.

Location (City, State, Year)	EPA Region	Variety	Total Rate (lbs ai/A)	PHI (days)	Residues (ppm) [1]		
					Acequinocyl	Acequinocyl-OH	Combined [2]
Clermont, FL, 2001	3	Midsweet	0.60	0	0.122, 0.078	<0.01, <0.01	0.127, 0.083
				7	0.038, 0.028	<0.01, <0.01	0.043, 0.033
				21	0.015, 0.014	<0.01, <0.01	0.020, 0.019
				35	<0.01, <0.01	<0.01, <0.01	0.01, 0.01
				49	0.011, <0.01	<0.01, <0.01	0.016, 0.01
Immokalee, FL, 2001	3	Parson Brown	0.60	7	0.023, 0.017	<0.01, <0.01	0.028, 0.022
DeLeon Springs, FL, 2001	3	Valencia	0.60	7	0.035, 0.045	<0.01, <0.01	0.040, 0.050
Clermont, FL, 2001	3	Valencia	0.60	7	0.078, 0.093	<0.01, <0.01	0.083, 0.098
Oviedo, FL, 2000	3	Pineapple	0.62	7	0.114, 0.106	<0.01, <0.01	0.119, 0.111
Mims, FL, 2000	3	Ambersweet	0.60	7	0.157, 0.168	0.012, 0.010	0.169, 0.178
Winter Garden, FL, 2001	3	Hamlin	0.60	7	0.025, 0.013	<0.01, <0.01	0.030, 0.018
St. Cloud, FL, 2001	3	Red Valencia	0.60	7	0.054, 0.037	<0.01, <0.01	0.059, 0.042
Raymondville, TX, 2001	6	N-33 Navels	0.60	7	0.052, 0.055	<0.01, <0.01	0.057, 0.060
Porterville, CA, 2001	10	Cutter Valencia	0.60	7	0.084, 0.048	<0.01, <0.01	0.089, 0.053
			2.36	7	0.270, 0.224	0.020, 0.015	0.290, 0.239
Richgrove, CA, 2001	10	Late Line Navels	0.60	7	0.063, 0.056	<0.01, <0.01	0.068, 0.059
Exeter, CA, 2001	10	Navel	0.60	7	0.114, 0.084	0.011, 0.011	0.125, 0.095

[1] The LOQ for each analyte is 0.01 ppm.
[2] Residues <0.01 ppm (<LOQ) were estimated to be 0.005 ppm for calculation of combined residues.

TABLE C.4. Summary of Residue Data for Orange Fruit from Orange Crop Field Trials with Acequinocyl.

Orange Matrix	Total Rate (lb a.i./A)	PHI (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [1]	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.60-0.62	7 [2]	24	0.013	0.168	0.163	0.066	0.042
Acequinocyl-OH Residues								
Fruit	0.60-0.62	7	24	<0.01 [3]	0.012	0.012	0.010	0.002
Combined Residues [4]								
Fruit	0.60-0.62	7	24	0.018	0.290	0.265	0.087	0.067

[1] HAFT = Highest Average Field Trial.
[2] The proposed PHI is 7 days.
[3] The LOQ for both analytes is 0.01 ppm in/on orange fruit.
[4] For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

D. CONCLUSION

The orange field trial residue data are adequate and reflect a maximum application rate of 0.62 lb ai/A of acequinocyl (FlC) and a 7-day PHI.

E. REFERENCES

45651604.der1
45651606.der1

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Grapefruit

Primary Evaluator: Sarah Levy, Chemist
Registration Action Branch 1 (RAB1)
Health Effects Division (HED; 7509C)

Approved by: George F. Kramer, Ph.D., Chemist
RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 20-NOV-2002). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45651607 Carringer, S. (2001) Magnitude of the Residue of Acequinocyl and its Metabolite in Grapefruit Raw Agricultural Commodities. Lab Project Number: TCI-01-004. Unpublished study prepared by Morse Laboratories, Inc. 188 p.

EXECUTIVE SUMMARY:

In a total of 6 field trials conducted in the U.S. during 2000 and 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications at a 21-day retreatment interval (RTI) to grapefruit trees during the later stages of fruit development at 0.30 lb ai/A/application, for a total of 0.60 lb ai/A/season. The number of crop field trials and geographic representation of the residue data on grapefruit are adequate. Duplicate grapefruit fruit samples were collected at 6 or 7 days after the last application in five trials and at 0, 7, 21, 35, and 49 days post-treatment in the residue decline trial.

Grapefruit fruit samples were stored frozen for a maximum of 111 days prior to analysis. The submitted stability data for acequinocyl and acequinocyl-OH in orange fruit stored frozen for up to 5 months (~150 days) are adequate (45651606.der1). Therefore, the storage intervals from the grapefruit field trials are supported by the available storage stability data.

Residues of acequinocyl and acequinocyl-OH in/on grapefruit matrices were determined by a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse #Meth-133, Revision 3), which was validated using oranges and found to be adequate for data collection (45651604.der1). For this method, residues are extracted by homogenizing with acetonitrile (ACN) or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica gel solid phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC and quantified by MS/MS detection in the positive ion mode. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on grapefruit fruit. The limit of detection (LOD) was not reported.

Following two applications of the 1.25 lb/gal FlC totaling 0.60 lb ai/A, combined residues of acequinocyl and acequinocyl-OH were 0.027-0.083 ppm in/on 12 grapefruit fruit samples harvested 6-7 days post-treatment. Average combined acequinocyl residues in/on grapefruit fruit samples declined from 0.140 ppm at 0 days post-treatment to 0.022 ppm at 49 days post-treatment.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The grapefruit field trial residue data are classified as scientifically acceptable under the conditions and parameters used in the study. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds, and pistachios in the United States. There are currently no food/feed uses or tolerances for acequinocyl.

TABLE A.1. Nomenclature of Test Compound and Metabolite

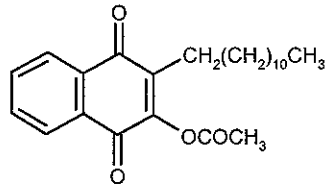
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	Kanemite™ 15 SC; 1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties

Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 acetone: >250 ethyl acetate: >250 xylene: >250 methanol: 6.1 n-heptane: 36 n-octanol: 29.2	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Temperatures and rainfall data were collected at each site. Air temperatures were within average historical values for the residue study period; however, rainfall amounts were lower than normal at the CA and FL sites. Irrigation was applied, as needed, to these sites.

TABLE B.1.1 Soil Characterization.

Study Location (City, State), Year	Soil Type
DeLeon Springs, FL, 2001	Fine Sand
Oviedo FL, 2000/2001	Sand
Clermont, FL, 2001	Sand
Raymondville, TX, 2001	Fine Sandy Loam
Porterville, CA, 2000/2001	Loam
Porterville, CA, 2001	Clay

TABLE B.1.2. Study Use Pattern on Grapefruit.

Location (City, State), Year	Application								Tank Mix Adjuvants
	Timing [1]	Formulation	Single Rate (lb a.i./A)	RTI [2] (days)	No. of Appl.	Method [3]	Volume (gal/A)	Total Rate (lb a.i./A)	
DeLeon Springs, FL, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	110-131	0.60	None
Oviedo FL, 2000/2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	69	0.60	None
Clermont, FL, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	168-171	0.60	None
Raymondville, TX, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	60	0.60	None
Porterville, CA, 2000/2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	243-247	0.60	None
Porterville, CA, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	59-60	0.60	None

[1] All applications were made to trees bearing fruit at the mid-mature to mature stage (4-7").
[2] RTI = Retreatment Interval
[3] All applications were made using ground equipment.

TABLE B.1.3. Trial Numbers and Geographical Locations

NAFTA Growing Region [1]	Total Grapefruit Trials		
	Submitted	Requested [2]	
		Canada	US
1	--	NA	--
2	--	NA	--
3	3	NA	3
4	--	NA	--
5	--	NA	--
6	1	NA	1
7	--	NA	--
8	--	NA	--
9	--	NA	--
10	2	NA	2
11	--	NA	--
12	--	NA	--
Total	6	NA	6

[1] Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the proposed use is for the US only.
[2] The number of requested grapefruit field trials when the proposed tolerance is for the citrus fruits crop group.
NA = not applicable.

B.2. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on grapefruit fruit was validated on orange fruit and found to be adequate for data collection (45651604.der1). A brief description of the method follows.

Residues are extracted by homogenizing with ACN or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica gel SPE cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on grapefruit fruit. The LOD was not reported.

C. RESULTS AND DISCUSSION

The number of crop field trials and geographic representation of the residue data on grapefruit is adequate according to the latest EPA Guidance.

Duplicate samples of whole fruit were collected from each trial at each interval and placed in frozen storage within 2.3 hours of sampling. Samples were stored (<0 C) at the field sites for 5-47 days prior to shipment by freezer truck to the analytical laboratory (Morse Laboratories, Sacramento, CA), where samples were stored at -20 C until analysis. The total frozen (-20 ± 5 C) storage intervals were 52-111 days for grapefruit fruit samples (Table C.2). The submitted stability data for acequinocyl and acequinocyl-OH in orange matrices stored frozen for up to 5 months (~150 days) are adequate (45651606.der1). Therefore, the storage intervals from the grapefruit field trials are supported by the available storage stability data.

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on grapefruit matrices was validated using oranges and found to be adequate for data collection (45651604.der1). Method recoveries from orange fruit, juice, dry pulp, and citrus oil samples fortified with each analyte at 0.01-25 ppm averaged 80-101% for acequinocyl and 70-116% for acequinocyl-OH. In the field trial analysis, concurrent method recoveries were 78 ± 4% for acequinocyl and 82 ± 13% for acequinocyl-OH from 4 grapefruit fruit control samples fortified separately with each analyte at 0.01-0.5 ppm (Table C.1). Apparent residues of acequinocyl and acequinocyl-OH were <0.01 ppm in/on all control grapefruit fruit samples. The LOQ for both analytes is 0.01 ppm in/on grapefruit fruit. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

In a total of 6 field trials conducted in the US during 2000 and 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications at a 21-day RTI to grapefruit trees during the later stages of fruit development at 0.30 lb ai/A/application, for a total of 0.60 lb ai/A/season. Duplicate grapefruit fruit samples were collected at 6 (one trial) or 7 (four trials) days after the last application in five trials and at 0, 7, 21, 35, and 49 days post-treatment in the residue decline trial.

Combined residues of acequinocyl and acequinocyl-OH were 0.026-0.082 ppm in/on 12 grapefruit fruit samples harvested 6-7 days post-treatment (Table C.3). Average combined acequinocyl residues in/on grapefruit fruit samples declined from 0.140 ppm at 0 days post-treatment to 0.022 ppm at 49 days post-treatment.

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.1 Concurrent Recovery Results from Grapefruit Field Trials Trial for HPLC/MS/MS Method Meth-133.

Grapefruit Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Whole Fruit	0.01-0.5	4	72-81	78 ± 4	73-100	82 ± 13

TABLE C.2. Summary of Freezer Storage Conditions

Grapefruit Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days) [1]
Fruit	-20 ± 5	52-111	~150 days

[1] The submitted stability data for acequinocyl and acequinocyl-OH residues in/on orange matrices stored frozen for up to 5 months (~150 days) are adequate (45651606.der1).

TABLE C.3. Grapefruit Fruit Residue Data from Grapefruit Field Trials with Acequinocyl.

Location (City, State, Year)	EPA Region	Variety	Total Rate (lbs ai/A)	PHI (days)	Residues (ppm) [1]		
					Acequinocyl	Acequinocyl-OH	Combined [2]
DeLeon Springs, FL, 2001	3	Texas Star Ruby Red	0.60	7	0.036, 0.035	<0.01, <0.01	0.041, 0.040
Oviedo FL, 2000/2001	3	Flame	0.60	0	0.142, 0.129	<0.01, <0.01	0.147, 0.134
				7	0.035, 0.044	<0.01, <0.01	0.040, 0.049
				21	0.034, 0.053	<0.01, <0.01	0.039, 0.058
				35	0.039, 0.028	<0.01, <0.01	0.044, 0.033
				49	<0.01, 0.029	<0.01, <0.01	0.010, 0.034
Clermont, FL, 2001	3	Flame	0.60	7	0.021, 0.025	<0.01, <0.01	0.026, 0.030
Raymondville, TX, 2001	6	Rio Red	0.60	7	0.029, 0.064	<0.01, <0.01	0.034, 0.069
Porterville, CA, 2000/2001	10	Mello Gold	0.60	6	0.041, 0.056	<0.01, <0.01	0.046, 0.061
Porterville, CA, 2001	10	Oro Blanco	0.60	7	0.077, 0.064	<0.01, <0.01	0.082, 0.069

[1] The LOQ for each analyte is 0.01 ppm.
[2] Residues <0.01 ppm (<LOQ) were estimated to be 0.005 ppm for calculation of combined residues.

TABLE C.4. Summary of Residue Data for Grapefruit Fruit from Grapefruit Crop Field Trials with Acequinocyl.

Grapefruit Matrix	Total Rate (lb a.i./A)	PHI (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [1]	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.60	6-7 [2]	12	0.021	0.077	0.071	0.044	0.018
Acequinocyl-OH Residues								
Fruit	0.60	6-7	12	<0.01 [3]	<0.01	<0.01	<0.01	NA
Combined Residues [4]								
Fruit	0.60	6-7	12	0.026	0.082	0.076	0.049	0.018

[1] HAFT = Highest Average Field Trial.
[2] The proposed PHI is 7 days.
[3] The LOQ for both analytes is 0.01 ppm in/on grapefruit fruit.
[4] For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

D. CONCLUSION

The grapefruit field trial residue data are adequate and reflect a maximum application rate of 0.60 lb ai/A (FlC) and a 7-day PHI.

E. REFERENCES

45651604.der1
45651606.der1

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Lemon

Primary Evaluator Sarah Levy, Chemist
Registration Action Branch 1 (RAB1)
Health Effects Division (HED; 7509C)

Approved by George F. Kramer, Ph.D., Chemist
RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 20-NOV-2002). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45651608 Carringer, S. (2001) Magnitude of the Residue of Acequinocyl and its Metabolite in Lemon Raw Agricultural Commodities. Lab Project Number: TCI-01-002. Unpublished study prepared by Morse Laboratories, Inc. 183 p.

EXECUTIVE SUMMARY:

In a total of 5 field trials conducted in the U.S. during 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications at a 21-day retreatment interval (RTI) to lemon trees during the later stages of fruit development at 0.299-0.302 lb ai/A/application, for a total of 0.598-0.603 lb ai/A/season. The number of crop field trials and geographic representation of the residue data on lemon are adequate. Duplicate lemon fruit samples were collected at 7 days after the last application in four trials and at 0, 7, 21, 35, and 49 days post-treatment in the residue decline trial.

Fruit samples were stored frozen for a maximum of 61 days prior to analysis. The submitted stability data for acequinocyl and acequinocyl-OH in oranges stored frozen for up to 5 months (~150 days) are adequate (45651606.der1). Therefore, the storage intervals from the lemon field trials are supported by the available storage stability data.

Residues of acequinocyl and acequinocyl-OH in/on lemons were determined by a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse #Meth-133, Revision 3), which was validated on oranges and found to be adequate for data collection (45651604.der1). For this method, residues are extracted by homogenizing with acetonitrile (ACN) or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica-gel solid-phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC and quantified by MS/MS detection in the positive ion mode. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on lemon fruit. The limit of detection (LOD) was not reported.

Following two applications of the 1.25 lb/gal FlC totaling 0.598-0.603 lb ai/A, combined residues of acequinocyl and acequinocyl-OH were 0.01-0.095 ppm in/on 10 lemon fruit samples harvested 7 days post-treatment. Average combined acequinocyl residues in/on lemon fruit samples declined from 0.278 ppm at 0 days post-treatment to 0.01 ppm at 49 days post-treatment.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The lemon field trial residue data are classified as scientifically acceptable under the conditions and parameters used in the study. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds, and pistachios in the United States. There are currently no food/feed uses or tolerances for acequinocyl.

TABLE A.1. Nomenclature of Test Compound and Metabolite

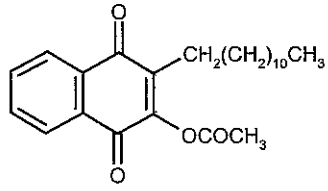
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	Kanemite™ 15 SC, 1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties

Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 ethyl acetate: >250 methanol: 6.1 n-octanol: 29.2 acetone: >250 xylene: >250 n-heptane: 36	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK$_a$)	no measurable pK$_a$	45434905
Octanol/water partition coefficient Log(K$_{OW}$)	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Temperatures and rainfall data were collected at each site, and were within average historical values for the residue study period. Rainfall was supplemented with irrigation as needed.

TABLE B.1.1 Soil Characterization.

Study Location (City, State), Year	Soil Type
Lakewood Park, FL, 2001	Sand
Ducor, CA, 2001	Clay
Terra Bella, CA, 2001	Clay
Richgrove, CA, 2001	Clay
Somis, CA, 2001	Clay

TABLE B.1.2. Study Use Pattern on Lemon.

Location (City, State) Year	Application								Tank Mix Adjuvants
	Timing [1]	Formulation	Single Rate (lb a.i./A)	RTI [2] (days)	No. of Appl.	Method [3]	Volume (gal/A)	Total Rate (lb a.i./A)	
Lakewood Park, FL, 2001	postemergence	1.25 lb/gal FlC	0.300	21	2	broadcast foliar	55	0.600	None
Ducor, CA, 2001	postemergence	1.25 lb/gal FlC	0.299	21	2	broadcast foliar	222-225	0.598	None
Terra Bella, CA, 2001	postemergence	1.25 lb/gal FlC	0.300	21	2	broadcast foliar	249	0.600	None
Richgrove, CA, 2001	postemergence	1.25 lb/gal FlC	0.299, 0.300	21	2	broadcast foliar	64	0.599	None
Somis, CA, 2001	postemergence	1.25 lb/gal FlC	0.301, 0.302	21	2	broadcast foliar	50	0.603	None

[1] All applications were made to trees during the later stages of fruit development (2-4" fruits).
[2] RTI = Retreatment Interval
[3] All applications were made using ground equipment.

TABLE B.1.3. Trial Numbers and Geographical Locations

NAFTA Growing Region [1]	Total Lemon Trials		
	Submitted	Requested	
		Canada	US
1	--	NA	--
2	--	NA	--
3	1	NA	1
4	--	NA	--
5	--	NA	--
6	--	NA	--
7	--	NA	--
8	--	NA	--
9	--	NA	--
10	4	NA	4
11	--	NA	--
12	--	NA	--
Total	5	NA	5

[1] Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the proposed use is for the US only.
NA = not applicable.

B.2. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on lemon fruit was validated using oranges and found to be adequate for data collection (45651604.der1). A brief description of the method follows.

Residues are extracted by homogenizing with ACN or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica gel SPE cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on lemon fruit. The LOD was not reported.

C. RESULTS AND DISCUSSION

The number of crop field trials and geographic representation of the residue data on lemons is adequate according to the latest EPA Guidance.

Duplicate samples of whole fruit were collected from each trial at each interval and placed in frozen storage within 2 hours of sampling. Samples were stored (<-5 C) at the field sites for 6-28 days prior to shipment by freezer truck to the analytical laboratory (Morse Laboratories, Sacramento, CA), where samples were stored at -20 C until analysis. The total frozen (-20 ± 5 C) storage intervals were 25-61 days for lemon fruit samples (Table C.2). The submitted stability data for acequinocyl and acequinocyl-OH residues in/on orange matrices stored frozen for up to 5 months (~150 days) are adequate (45651606.der1). Therefore, the storage intervals from the lemon field trials are supported by the available storage stability data.

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on lemon matrices was validated on oranges and found to be adequate for data collection (45651604.der1). Method recoveries from orange fruit, juice, dry pulp, and citrus oil samples fortified with each analyte at 0.01- 25 ppm averaged 80-101% for acequinocyl and 70-116% for acequinocyl-OH. In the field trial analysis, concurrent method recoveries were 94 ± 9% for acequinocyl and 84 ± 9% for acequinocyl-OH from 4 lemon fruit control samples fortified separately with each analyte at 0.01-0.5 ppm (Table C.1). Apparent residues of acequinocyl and acequinocyl-OH were <0.01 ppm in/on all control lemon fruit samples. The LOQ for both analytes is 0.01 ppm in/on lemon fruit. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

In a total of 5 field trials conducted in the U.S. during 2001, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications at a 21-day RTI to lemon trees during the later stages of fruit development at 0.299-0.302 lb ai/A/application, for a total of 0.598-0.603 lb ai/A/season. Duplicate lemon fruit samples were collected at 7 days after the last application in four trials and at 0, 7, 21, 35, and 49 days post-treatment in the residue decline trial.

Combined residues of acequinocyl and acequinocyl-OH were 0.01-0.095 ppm in/on 10 lemon fruit samples harvested 7 days post-treatment (Table C.3). Average combined acequinocyl residues in/on lemon fruit samples declined from 0.278 ppm at 0 days post-treatment to 0.01 ppm at 49 days post-treatment.

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.1 Concurrent Recovery Results from Lemon Field Trials Trial for HPLC/MS/MS Method Meth-133.

Lemon Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Whole Fruit	0.01-0.5	4	81-102	94 ± 9	78-98	84 ± 9

TABLE C.2. Summary of Freezer Storage Conditions

Lemon Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days)[1]
Fruit	-20 ± 5	25-61	~150 days

[1] The submitted stability data for acequinocyl and acequinocyl-OH residues in/on orange matrices stored frozen for up to 5 months (~150 days) are adequate (45651606.der1).

TABLE C.3. Lemon Fruit Residue Data from Lemon Field Trials with Acequinocyl.

Location (City, State, Year)	EPA Region	Variety	Total Rate (lbs ai/A)	PHI (days)	Residues (ppm)[1]		
					Acequinocyl	Acequinocyl-OH	Combined[2]
Lakewood Park, FL, 2001	3	Bearrs	0.600	7	0.079, 0.079	0.012, 0.010	0.091, 0.089
Ducor, CA, 2001	10	Lisbon	0.598	0	0.234, 0.278	0.021, 0.024	0.255, 0.302
				7	0.047, 0.051	0.021, 0.020	0.068, 0.071
				21	<0.01, <0.01	<0.01, <0.01	0.01, 0.01
				35	<0.01, <0.01	<0.01, <0.01	0.01, 0.01
				49	<0.01, <0.01	<0.01, <0.01	0.01, 0.01
Terra Bella, CA, 2001	10	Lisbon	0.600	7	0.064, 0.068	0.025, 0.027	0.089, 0.095
Richgrove, CA, 2001	10	Lisbon	0.599	7	0.034, 0.028	0.030, 0.025	0.064, 0.053
Somis, CA, 2001	10	Eureka	0.603	7	<0.01, <0.01	<0.01, 0.011	0.011, 0.016

[1] The LOQ for each analyte is 0.01 ppm.
[2] Residues <0.01 ppm (<LOQ) were estimated to be 0.005 ppm for calculation of combined residues.

TABLE C.4. Summary of Residue Data for Lemon Fruit from Lemon Crop Field Trials with Acequinocyl.

Lemon Matrix	Total Rate (lb a.i./A)	PHI (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [1]	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.598-0.603	7 [2]	10	<0.01 [3]	0.079	0.079	0.046	0.028
Acequinocyl-OH Residues								
Fruit	0.598-0.603	7	10	<0.01	0.030	0.028	0.019	0.009
Combined Residues [4]								
Fruit	0.598-0.603	7	10	0.01	0.095	0.092	0.065	0.030

[1] HAFT = Highest Average Field Trial.
[2] The proposed PHI is 7 days.
[3] The LOQ for both analytes is 0.01 ppm in/on lemon fruit.
[4] For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

D. CONCLUSION

The lemon field trial residue data are adequate and reflect a maximum application rate of 0.603 lb ai/A of acequinocyl (FlC) and a 7-day PHI.

E. REFERENCES

45651606.der1
45651604.der1

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Apple

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 20-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45651604 Carringer, S. (2001) Magnitude of the Residue of Acequinocyl and its Metabolite in Apple Raw Agricultural and Processed Commodities. Lab Project Number: TCI-00-001. Unpublished study prepared by Morse Laboratories, Inc. 397 p.

EXECUTIVE SUMMARY:

In a total of 12 field trials conducted throughout the U.S. during 2000, acequinocyl (1.25 lb/gal FlC) was applied to apple trees as two broadcast foliar applications at 0.298-0.308 lb ai/A/application, for a total of 0.596-0.615 lb ai/A/season. The applications were made during the later stages of fruit development at a 21- or 22-day retreatment interval (RTI). The number of crop field trials and geographic representation of the residue data on apple are adequate. Duplicate samples of apple fruit were collected at 13-15 days after the last application in eleven trials and at 0, 7, 14, and 21 days post-treatment in one residue decline trial.

Apple fruit samples were stored frozen for a maximum of 144 days prior to analysis. This storage interval is supported by the available stability data, which indicate that acequinocyl and acequinocyl-OH residues are stable in frozen apples for up to 5 months (45651604.der2).

Residues of acequinocyl and acequinocyl-OH in/on apple matrices were determined by a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse #Meth-133, Revision 3), which was validated and found to be adequate for data collection (45651604.der1). For this method, residues are extracted by homogenizing with acetonitrile (ACN) or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica-gel solid-phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC and quantified by MS/MS detection in the positive ion mode. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on apple fruit. The limit of detection (LOD) was not reported.

Following two applications of the 1.25 lb/gal FlC totaling 0.596-0.615 lb ai/A, combined residues of acequinocyl and acequinocyl-OH were 0.024-0.225 ppm in/on 24 apple fruit samples harvested 13-15 days post-treatment. Average combined acequinocyl residues in/on apple fruit samples declined from 0.224 ppm at 0 days post-treatment to 0.117 ppm at 21 days post-treatment.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the apple field trial residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

TABLE A.1. Nomenclature of Test Compound and Metabolite

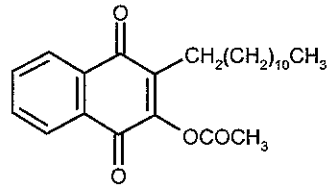
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties

Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 ethyl acetate: >250 methanol: 6.1 n-octanol: 29.2 acetone: >250 xylene: >250 n-heptane: 36	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Temperatures and rainfall data were collected at each site, and were within average historical values for the residue study period. Rainfall was supplemented with irrigation as needed.

TABLE B.1.1 Soil Characterization.

Study Location (City, State), Year	Soil Type
Dundee, NY, 2000	Gravelly Loam
Sodus, NY, 2000	Cobbly Loam
New Smithville, PA, 2000	Silt Loam
Monetta, SC, 2000	Sandy Loam
Dix, IL, 2000	Silt Loam
Conklin, MI, 2000	Loam
Perry, UT, 2000	Gravelly Loam
Terra Bella, CA, 2000	Loam
Parma, ID, 2000	Sandy Loam
Caldwell, ID, 2000	Sandy Loam
Payette, ID, 2000	Loam
Ephrata, WA, 2000	Sandy Loam

TABLE B.1.2. Study Use Pattern on Apple.

Location (City, State, Year)	Application								Tank Mix Adjuvants
	Timing [1]	Formulation	Single Rate (lb a.i./A)	RTI [2] (days)	No. of Appl.	Method [3]	Volume (gal/A)	Total Rate (lb a.i./A)	
Dundee, NY, 2000	postemergence	1.25 lb/gal FlC	0.298	21	2	broadcast foliar	50	0.596	None
Sodus, NY, 2000	postemergence	1.25 lb/gal FlC	0.300 1.21-1.22	21	2	broadcast foliar	200-202	0.600 2.43	None
New Smithville, PA, 2000	postemergence	1.25 lb/gal FlC	0.300	21	2	broadcast foliar	123	0.600	None
Monetta, SC, 2000	postemergence	1.25 lb/gal FlC	0.303-0.307	22	2	broadcast foliar	68-70	0.610	None
Dix, IL, 2000	postemergence	1.25 lb/gal FlC	0.301-0.302	21	2	broadcast foliar	68	0.603	None
Conklin, MI, 2000	postemergence	1.25 lb/gal FlC	0.301	21	2	broadcast foliar	160-163	0.602	None
Perry, UT, 2000	postemergence	1.25 lb/gal FlC	0.299-0.300	21	2	broadcast foliar	52-56	0.599	None
Terra Bella, CA, 2000	postemergence	1.25 lb/gal FlC	0.302	21	2	broadcast foliar	284	0.604	None
Parma, ID, 2000	postemergence	1.25 lb/gal FlC	0.307-0.308	21	2	broadcast foliar	69-73	0.615	None
Caldwell, ID, 2000	postemergence	1.25 lb/gal FlC	0.301	21	2	broadcast foliar	205-207	0.602	None
Payette, ID, 2000	postemergence	1.25 lb/gal FlC	0.305-0.307	21	2	broadcast foliar	102	0.612	None
Ephrata, WA, 2000	postemergence	1.25 lb/gal FlC	0.300	21	2	broadcast foliar	65-67	0.600	None

[1] All applications were made to trees at the 2-4" fruit stage.
[2] RTI = Retreatment Interval
[3] All applications were made using ground equipment.

TABLE B.1.3. Trial Numbers and Geographical Locations

NAFTA Growing Region [1]	Total Apple Trials		
	Submitted	Requested [2]	
		Canada	US
1	3	NA	3
2	1	NA	1
3	--	NA	--
4	--	NA	--
5	2	NA	2
6	--	NA	--
7	--	NA	--
8	--	NA	--
9	1	NA	1
10	1	NA	1
11	4	NA	4
12	--	NA	--
Total	12	NA	12

[1] Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the proposed use is for the US only.
[2] The number of requested apple field trials when the proposed tolerance is for the pome fruits crop group.
NA = not applicable.

B.2. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on apple fruit was validated and found to be adequate for data collection (45651604.der1). A brief description of the method follows.

Residues are extracted by homogenizing with ACN or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica gel SPE cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on apple fruit. The LOD was not reported.

C. RESULTS AND DISCUSSION

The number of crop field trials and geographic representation of the residue data on apples is adequate according to the latest EPA Guidance.

Duplicate samples of whole fruit were collected from each trial at each interval and placed in frozen storage within 3 hours of sampling. Samples were stored (<-5 C) at the field sites for 0-48 days prior to shipment by freezer truck to the analytical laboratory (Morse Laboratories, Sacramento, CA), where samples were stored at -20 C until analysis. The total frozen (-20 ± 5 C) storage intervals were 77-144 days for apple fruit samples (Table C.2). These storage intervals are supported by the available stability data, which indicate that acequinocyl and acequinocyl-OH residues are stable in frozen apples for up to 5 months (45651604.der2).

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on apple matrices was validated and found to be adequate for data collection (45651604.der1). Method recoveries from apple fruit, juice, and wet pomace samples fortified with each analyte at 0.01 or 0.5 ppm averaged 84-96% for acequinocyl and 76-81% for acequinocyl-OH. In the apple field trial analyses, concurrent method recoveries were 96 ± 13% for acequinocyl and 78 ± 5% for acequinocyl-OH from 6 apple fruit control samples fortified separately with each analyte at 0.01-2.0 ppm (Table C.1). Apparent residues of acequinocyl and acequinocyl-OH were <0.01 ppm in/on all control apple fruit samples. The LOQ for both analytes is 0.01 ppm in/on apple fruit. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

In a total of 12 field trials conducted throughout the U.S. during 2000, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to apple trees at a 21- or 22-day RTI during the later stages of fruit development at 0.298-0.308 lb ai/A/application, for a total of 0.596-0.615 lb ai/A/season. Duplicate apple fruit samples were collected at 13-15 days after the last application in eleven trials and at 0, 7, 14, and 21 days post-treatment in the residue decline trial.

Combined residues of acequinocyl and acequinocyl-OH were 0.024-0.225 ppm in/on 24 apple fruit samples harvested 13-15 days post-treatment (Table C.3). Average combined acequinocyl residues in/on apple fruit samples declined from 0.224 ppm at 0 days post-treatment to 0.117 ppm at 21 days post-treatment.

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.1 Concurrent Recovery Results from Apple Field Trials Trial for HPLC/MS/MS Method Meth-133.

Apple Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Whole Fruit	0.01-2.0	6	82-115	96 ± 13	70-83	78 ± 5

TABLE C.2. Summary of Freezer Storage Conditions

Apple Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days) [1]
Fruit	-20 ± 5	77-144	150

[1] The submitted stability data for acequinocyl and acequinocyl-OH residues in apple matrices stored frozen for up to 5 months (~150 days) are adequate (45651604.der2).

TABLE C.3. Apple Fruit Residue Data from Apple Field Trials with Acequinocyl.

Location (City, State, Year)	EPA Region	Variety	Total Rate (lbs ai/A)	PHI (days)	Residues (ppm) [1]		
					Acequinocyl	Acequinocyl-OH	Combined [2]
Dundee, NY, 2000	1	Empire	0.596	0	0.240, 0.199	<0.01, <0.01	0.245, 0.204
				7	0.202, 0.176	<0.01, <0.01	0.207, 0.181
				14	0.128, 0.139	<0.01, <0.01	0.133, 0.144
				21	0.134, 0.090	<0.01, <0.01	0.139, 0.095
Sodus, NY, 2000	1	McIntosh	0.600	14	0.056, 0.042	<0.01, <0.01	0.061, 0.047
			2.43	14	0.331, 0.350	<0.01, <0.01	0.336, 0.355
New Smithville, PA, 2000	1	Red Delicious	0.600	15	0.156, 0.160	<0.01, <0.01	0.161, 0.165
Monetta, SC, 2000	2	Granny Smith	0.610	14	0.019, 0.026	<0.01, <0.01	0.024, 0.031
Dix, IL, 2000	5	Jonathan	0.603	13	0.073, 0.049	<0.01, <0.01	0.078, 0.054
Conklin, MI, 2000	5	McIntosh	0.602	14	0.054, 0.022	<0.01, <0.01	0.059, 0.027
Perry, UT, 2000	9	Red Delicious	0.599	14	0.043, 0.053	<0.01, <0.01	0.048, 0.058
Terra Bella, CA, 2000	10	Fuji	0.604	14	0.024, 0.050	<0.01, <0.01	0.029, 0.055
Parma, ID, 2000	11	Rome	0.615	14	0.109, 0.116	<0.01, <0.01	0.114, 0.121
Caldwell, ID, 2000	11	Red Delicious	0.602	14	0.047, 0.034	<0.01, <0.01	0.052, 0.039
Payette, ID, 2000	11	Law Rome	0.612	14	0.193, 0.220	<0.01, <0.01	0.198, 0.225
Ephrata, WA, 2000	11	Red Delicious	0.600	14	0.121, 0.114	<0.01, <0.01	0.126, 0.119

[1] The LOQ for each analyte is 0.01 ppm. The residues are expressed in terms of each analyte.
[2] Residues <0.01 ppm (<LOQ) were estimated to be 0.005 ppm for calculation of combined residues and average residues.

TABLE C.4. Summary of Residue Data for Apple Fruit from Apple Crop Field Trials with Acequinocyl.								
Apple Matrix	Total Rate (lb a.i./A)	PHI (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [1]	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.596-0.615	13-15 [2]	24	0.019	0.220	0.207	0.085	0.058
Acequinocyl-OH Residues								
Fruit	0.596-0.615	13-15	24	<0.01 [3]	<0.01	<0.01	<0.01	0
Combined Residues [4]								
Fruit	0.596-0.615	13-15	24	0.024	0.225	0.212	0.109	0.089

[1] HAFT = Highest Average Field Trial.
[2] The proposed PHI is 14 days; 20 of 24 samples were harvested at 14-day PHI.
[3] The LOQ for both analytes is 0.01 ppm in/on apple fruit.
[4] For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

D. CONCLUSION

The apple field trial residue data are adequate and reflect a maximum application rate of 0.615 lb ai/A of acequinocyl (FlC) and a 14-day PHI.

E. REFERENCES

45651604.der1
45651604.der2

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Pear

Primary Evaluator: Sarah Levy, Chemist
Registration Action Branch 1 (RAB1)
Health Effects Division (HED; 7509C)

Approved by: George F. Kramer, Ph.D., Chemist
RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 20-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45651605 Carringer, S. (2001) Magnitude of the Residue of Acequinocyl and its Metabolite in Pear Raw Agricultural Commodities. Lab Project Number: TCI-00-002. Unpublished study prepared by Morse Laboratories, Inc. 196 p.

EXECUTIVE SUMMARY:

In a total of 6 field trials conducted in the U.S. during 2000, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications at a 21- or 22-day retreatment interval (RTI) to pear trees during the later stages of fruit development at 0.292-0.306 lb ai/A/application, for a total of 0.591-0.610 lb ai/A/season. The number of crop field trials and geographic representation of the residue data on pear are adequate. Duplicate pear fruit samples were collected at 14 days after the last application in five trials and at 0, 7, 14, and 21 days post-treatment in the residue decline trial.

Pear fruit samples were stored frozen for a maximum of 144 days prior to analysis. This storage interval is supported by the available stability data, which indicate that acequinocyl and acequinocyl-OH are stable in frozen apples for up to 5 months (45651604.der2).

Residues of acequinocyl and acequinocyl-OH in/on pears were determined by a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse #Meth-133, Revision 2), which was validated using apples and found to be adequate for data collection (45651604.der1). For this method, residues are extracted by homogenizing with acetonitrile (ACN) or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica-gel solid-phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC and quantified by MS/MS detection in the positive ion mode. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on fruit. The limit of detection (LOD) was not reported.

Following two applications of the 1.25 lb/gal FlC totaling 0.591-0.610 lb ai/A, combined residues of acequinocyl and acequinocyl-OH were 0.01-0.048 ppm in/on 12 pear fruit samples

harvested 14 days post-treatment. Average combined acequinocyl residues in/on pear fruit samples declined from 0.163 ppm at 0 days post-treatment to 0.029 ppm at 21 days post-treatment.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the pear field trial residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

TABLE A.1. Nomenclature of Test Compound and Metabolite

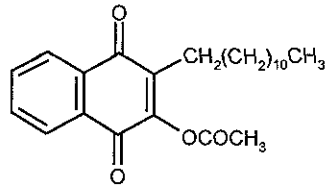
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties

Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 acetone: >250 ethyl acetate: >250 xylene: >250 methanol: 6.1 n-heptane: 36 n-octanol: 29.2	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Temperatures and rainfall data were collected at each site, and were within average historical values for the residue study period. Rainfall was supplemented with irrigation as needed.

TABLE B.1.1 Soil Characterization.

Study Location (City, State), Year	Soil characteristics
Sodus, NY, 2000	Gravelly Loam
Porterville, CA, 2000	Loam
Lindsay, CA, 2000	Loam
Greenleaf, ID, 2000	Silt Loam
Ephrata, WA, 2000	Silt Loam
Fruitland, ID, 2000	Silt Loam

TABLE B.1.2. Study Use Pattern on Pear.

Location (City, State), Year	Application								Tank Mix Adjuvants
	Timing [1]	Formulation	Single Rate (lb a.i./A)	RTI [2] (days)	No. of Appl.	Method [3]	Volume (gal/A)	Total Rate (lb a.i./A)	
Sodus, NY, 2000	postemergence	1.25 lb/gal FlC	0.301, 0.304	22	2	broadcast foliar	201-203	0.605	None
Porterville, CA, 2000	postemergence	1.25 lb/gal FlC	0.292, 0.299	21	2	broadcast foliar	58-60	0.591	None
Lindsay, CA, 2000	postemergence	1.25 lb/gal FlC	0.300	21	2	broadcast foliar	252	0.600	None
Greenleaf, ID, 2000	postemergence	1.25 lb/gal FlC	0.304, 0.306	22	2	broadcast foliar	65-67	0.610	None
Ephrata, WA, 2000	postemergence	1.25 lb/gal FlC	0.300	21	2	broadcast foliar	59	0.600	None
Fruitland, ID, 2000	postemergence	1.25 lb/gal FlC	0.301,0.302	21	2	broadcast foliar	100	0.603	None

[1] All applications were made to trees during the later stages of fruit development (fruit 2-4").
[2] RTI = Retreatment Interval
[3] All applications were made using ground equipment.

TABLE B.1.3. Trial Numbers and Geographical Locations

NAFTA Growing Region[1]	Total Pear Trials		
	Submitted	Requested[2]	
		Canada	US
1	1	NA	1
2	--	NA	--
3	--	NA	--
4	--	NA	--
5	--	NA	--
6	--	NA	--
7	--	NA	--
8	--	NA	--
9	--	NA	--
10	2	NA	2
11	3	NA	3
12	--	NA	--
Total	6	NA	6

[1] Regions 13-21 and 1A, 5A, 5B, and 7A were not included as the proposed use is for the US only.
[2] The number of requested pear field trials when the proposed tolerance is for the pome fruits crop group.
NA = not applicable.

B.2. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-133, Revision 2) for determining residues of acequinocyl and acequinocyl-OH in/on pear matrices was validated using apple matrices and found to be adequate for data collection (45651604.der1). A brief description of the method used for pear fruit follows.

Residues are extracted by homogenizing with ACN or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica gel SPE cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on fruit. The LOD for all analytes in/on all matrices was not reported.

C. RESULTS AND DISCUSSION

The number of crop field trials and geographic representation of the residue data on pears is adequate according to the latest EPA Guidance.

Duplicate samples of whole fruit were collected from each trial at each interval and placed in frozen storage within 2 hours of sampling. Samples were stored (<-5 C) at the field sites for 2-35 days prior to shipment by freezer truck to the analytical laboratory (Morse Laboratories, Sacramento, CA), where samples were stored at -20 C until analysis. The total frozen (-20 ± 5 C) storage intervals were 107-144 days for pear fruit samples (Table C.2). These intervals are supported by the available storage data, indicating that acequinocyl and acequinocyl-OH are stable in frozen apples for up to 5 months (45651604.der2).

The HPLC/MS/MS method (Morse #Meth-133, Revision 2) for determining residues of acequinocyl and acequinocyl-OH in/on pear matrices was validated using apples and found to be adequate for data collection (45651604.der1). Method recoveries from apple fruit, juice, and wet pomace samples fortified with each analyte at 0.01 or 0.5 ppm averaged 84-96% for acequinocyl and 76-81% for acequinocyl-OH. In the pear field trial analysis, concurrent method recoveries were 97 ± 3% for acequinocyl and 85 ± 3% for acequinocyl-OH from 3 pear fruit control samples fortified separately with each analyte at 0.01-0.5 ppm (Table C.1). Apparent residues of acequinocyl and acequinocyl-OH residues were <0.01 ppm in/on all control pear fruit samples. The LOQ for both analytes is 0.01 ppm in/on pear fruit. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

In a total of 6 field trials conducted in throughout the U.S. during 2000, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications at 0.292-0.306 lb ai/A/application, for a total of 0.591-0.610 lb ai/A/season. The applications were made during the later stages of fruit development at a 21- or 22-day RTI. Duplicate pear fruit samples were collected at 14 days after the last application in five of the trials and at 0, 7, 14, and 21 days post-treatment in the residue decline trial.

Combined residues of acequinocyl and acequinocyl-OH were 0.01-0.048 ppm in/on 12 pear fruit samples harvested 14 days post-treatment (Table C.3). Average combined acequinocyl residues in/on pear fruit samples declined from 0.163 ppm at 0 days post-treatment to 0.029 ppm at 21 days post-treatment.

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.1. Concurrent Recovery Results from Pear Field Trials Trial for HPLC/MS/MS Method Meth-133.

Pear Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Fruit	0.01-0.5	3	93-99	97 ± 3	82-87	85 ± 3

TABLE C.2. Summary of Freezer Storage Conditions

Pear Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days) [1]
Fruit	-20 ± 5	107-144	~150 days

[1] The submitted stability data for acequinocyl and acequinocyl-OH residues in/on apple matrices stored frozen for up to 5 months (~150 days) was adequate (45651604.der2).

TABLE C.3. Pear Fruit Residue Data from Pear Field Trials with Acequinocyl.

Location (City, State, Year)	EPA Region	Variety	Total Rate (lbs ai/A)	PHI (days)	Residues (ppm) [1]		
					Acequinocyl	Acequinocyl-OH	Combined [2]
Sodus, NY, 2000	1	Bartlett	0.605	0	0.178, 0.137	<0.01, <0.01	0.183, 0.142
				7	0.069, 0.083	<0.01, <0.01	0.074, 0.088
				14	0.036, 0.043	<0.01, <0.01	0.041, 0.048
				21	0.021, 0.026	<0.01, <0.01	0.026, 0.031
Porterville, CA, 2000	10	Bosc	0.591	14	0.012, 0.010	<0.01, <0.01	0.017, 0.015
Lindsay, CA, 2000	10	Olympia	0.600	14	0.042, 0.024	<0.01, <0.01	0.047, 0.029
Greenleaf, ID, 2000	11	Bartlett	0.610	14	0.025, 0.030	<0.01, <0.01	0.029, 0.035
Ephrata, WA, 2000	11	Bartlett	0.600	14	0.020, 0.017	<0.01, <0.01	0.025, 0.022
Fruitland, ID, 2000	11	Bartlett	0.603	14	<0.01, <0.01	<0.01, <0.01	0.01, 0.01

[1] The LOQ for each analyte is 0.01 ppm. The residues are expressed in terms of each analyte.
[2] Residues <0.01 ppm (<LOQ) were estimated to be 0.005 ppm for calculation of combined residues and average residues.

TABLE C.4. Summary of Residue Data for Pear Fruit from Pear Crop Field Trials with Acequinocyl.								
Pear Matrix	Total Rate (lb a.i./A)	PHI (days)	No of samples	Residue Levels (ppm)				
				Min.	Max.	HAFT [1]	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.591-0.610	14 [2]	12	<0.01	0.043	0.040	0.022	0.013
Acequinocyl-OH Residues								
Fruit	0.591-0.610	14	12	<0.01 [3]	<0.01	<0.01	0.01	NA
Combined Residues [4]								
Fruit	0.591-0.610	14	12	0.01	0.048	0.045	0.027	0.013

1 HAFT = Highest Average Field Trial.
2 The proposed PHI is 14 days.
3 The LOQ for both analytes is 0.01 ppm in/on pear fruit.
4 For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

D. CONCLUSION

The pear field trial residue data are adequate and reflect a maximum application rate of 0.610 lb ai/A of acequinocyl (FIC) and a 14-day PHI.

E. REFERENCES

45651604.der2
45651604.der1

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/006329/Arvesta Corporation and Agro-Kanesho Co., Ltd.
DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Crop Field Trial - Strawberry

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

STUDY REPORTS:

MRID No. 45896801. Carringer, S. (2002) Magnitude of the Residue of Acequinocyl and its Metabolite in Strawberry Raw Agricultural Commodities: Lab Project Number: TCI-02-059. Unpublished study prepared by Morse Laboratories, Inc. 208 pages.

EXECUTIVE SUMMARY:

Arvesta Corporation and Agro-Kanesho Co., Ltd. have submitted field trial data for acequinocyl on strawberries. Eight trials were conducted encompassing EPA Regions I (NY; 1 trial), II (SC; 1 trial), III (FL; 1 trial), V (MI; 1 trial), X (CA; 3 trials), and XII (OR; 1 trial) during the 2002 growing season. The number and locations of field trials are in accordance with OPPTS Guideline 860.1500.

At each trial location, two plots were established (the first plot was the untreated control, the second plot received two applications of Kanemite™ 15 soluble concentrate (SC) (1.25 lb/gal flowable concentrate (FlC)) at the maximum expected label use rate of ~0.30 lb ai/A, for a total of ~0.60 lb ai/A/season). The applications were made at 22 days and 1 day before harvest. An adjuvant was not added to the spray mixture for all applications. Strawberries were harvested at a 1-day PHI, with the exception of one trial site in Region X where samples were also collected at 0, 4, 7, and 14-day PHIs to determine if residues increase or decrease with longer PHIs.

Residues of acequinocyl and acequinocyl-OH in/on strawberry matrices were determined by a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse #Meth-133, Revision 3), which was validated and found to be adequate for data collection (45651604.der1). For this method, residues are extracted by homogenizing with acetonitrile (ACN) or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica-gel solid-phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC and quantified by MS/MS detection in the positive ion mode. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on strawberry fruit. The limit of detection (LOD) was not reported.

Acequinocyl residues have been shown to be stable for the duration of storage that occurred during the conduct of this study. The results from these trials show that combined residues of acequinocyl and acequinocyl-OH were 0.145-0.350 ppm in/on 16 strawberry fruit samples harvested 1 day post-treatment. Average combined acequinocyl residues in/on strawberry

samples declined from 0.427 ppm at 0 days post-treatment to 0.022 ppm at 14 days post-treatment. Residue decline data show that acequinocyl residues appear to decrease in strawberries with increasing pre-harvest intervals.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the strawberry field trial residue data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D290204].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 SC , 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds, and pistachios in the United States. There are currently no food/feed uses or tolerances for acequinocyl.

TABLE A.1. Nomenclature of Test Compound and Metabolite

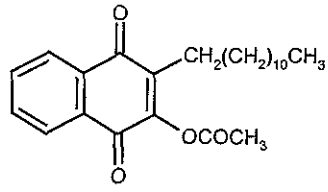
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	Kanemite™ 15 SC; 1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound

Parameter	Value	Reference
Melting point/range	59.6 °C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 acetone: >250 ethyl acetate: >250 xylene: >250 methanol: 6.1 n-heptane: 36 n-octanol: 29.2	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK_a)	no measurable pK_a	45434905
Octanol/water partition coefficient Log(K_{OW})	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1 Trial Site Conditions

Trial Identification (City, State/Year)	Soil characteristics				Meteorological data[1]	
	Type	%OM	pH	CEC meq/g	Overall Monthly rainfall range (inches)	Overall T°C range
Penn Yan, NY/2002	Fine sandy loam	NS*	NS	NS	2.9	38008
Johnston, SC/2002	Sandy loam				1.5	37951
Dover, FL/2002	Loamy fine sand				0.72	14-27
Kent City, MI/2002	Loamy sand				2.8	14-24
Porterville, CA/2002	Sandy loam				1.44 0.00	7-21 14-21
Exeter, CA/2002	Loam				0.30 0.24	9-24 11-28
Hughson, CA/2002	Sandy loam				1.43 0.2	7-19 10-23
Forest Grove, OR/2002	Silt loam				1.16 1.2	45124

* NS = Not Specified.
[1] The two separate entries for a field trial represent data for two consecutive months.

The actual temperature recordings are within average historical values for the residue study period. The actual rainfall average was within the historical rainfall average. Irrigation was used to supplement as needed.

TABLE B.1.2. Study Use Pattern.

Location (City, State/Year)	EP[1]	Application					
		Method/Timing	Vol, GPA[2]	Rate (lb a.i./A)	RTI,[3] (days)	Total Rate (lb a.i./A)	Tank Mix Adjuvants
Penn Yan, NY/ 2002	15% SC; 1.25 lb/gal FlC	Backpack Sprayer/ Postemergence	148	0.295	20	0.588	None
			146.8	0.293			
Johnston, SC/2002	15% SC; 1.25 lb/gal FlC	Backpack Sprayer/ Postemergence	114.2	0.296	21	0.6	None
			108.3	0.304			
Dover, FL/2002	15% SC; 1.25 lb/gal FlC	Backpack Sprayer/ Postemergence	134.4	0.296	21	0.6	None
			138.3	0.304			
Kent City, MI/2002	15% SC; 1.25 lb/gal FlC	Tractor-mounted Sprayer/ Postemergence	102.8	0.299	21	0.591	None
			100.4	0.292			
Porterville, CA/2002	15% SC; 1.25 lb/gal FlC	Tractor-mounted Backpack Sprayer/ Postemergence	100.8	0.303	20	0.6	None
			99	0.297			

TABLE B.1.2. Study Use Pattern.

Location (City, State/Year)	EP[1]	Application					
		Method/Timing	Vol, GPA[2]	Rate (lb a.i./A)	RTI,[3] (days)	Total Rate (lb a.i./A)	Tank Mix Adjuvants
Exeter, CA/2002	15% SC; 1.25 lb/gal FlC	Tractor-mounted Boom Sprayer/ Postemergence	101.9	0.302	21	0.603	None
			100.9	0.301			
Hughson, CA/2002	15% SC; 1.25 lb/gal FlC	Backpack Sprayer/ Postemergence	108.6	0.294	21	0.587	None
			108.5	0.293			
Forest Grove, OR/2002	15% SC; 1.25 lb/gal FlC	Backpack Sprayer/ Postemergence	132.2	0.301	21	0.602	None
			137	0.301			

[1]EP = End-use Product
[2] Gallons per acre
[3] Retreatment Interval

TABLE B.1.3. Trial Numbers and Geographical Locations

NAFTA Growing Region	Strawberry		
	Submitted	Requested	
		Canada	US
1	1		1
2	1		1
3	1		1
4			
5	1		1
5B			
6			
7			
8			
9			
10	3		3
11			
12	1		1
13			
14			
15			
16			
17			
18			
19			
20			
21			
Total	8		8

B.2. Sample Handling and Preparation

All strawberry RAC samples were collected and placed in freezer storage within 2.5 hours of collection and maintained frozen (≤-2°C) at the field test sites for 2-34 days until shipped to Morse Laboratories, Inc. The samples were shipped frozen via freezer trucks to Morse Laboratories, Inc. for analysis.

B.3. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on strawberry fruit was validated and found to be adequate for data collection (45651604.der1). A brief description of the method follows.

Residues are extracted by homogenizing with ACN or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica gel SPE cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on strawberry fruit. The LOD was not reported.

C. RESULTS AND DISCUSSION

The number of crop field trials and geographic representation of the residue data on strawberries is adequate according to the latest EPA Guidance.

Duplicate samples of whole fruit were collected from each trial at each interval and placed in frozen storage within 2.5 hours of sampling. Samples were stored (≤-2°C) at the field sites for 2-34 days prior to shipment by freezer truck to the analytical laboratory (Morse Laboratories, Sacramento, CA), where samples were stored frozen until analysis. The total frozen storage intervals were 51-110 days for strawberry fruit samples (Table C.2). The submitted stability data for acequinocyl and acequinocyl-OH in orange matrices stored frozen for up to 5 months (~150 days) are adequate (45651606.der1).

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on fruit matrices was validated using oranges and found to be adequate for data collection (45651604.der1). Method recoveries from orange fruit, juice, dry pulp, and citrus oil samples fortified with each analyte at 0.01-25 ppm averaged 80-101% for acequinocyl and 70-116% for acequinocyl-OH. In the field trial analysis, concurrent method recoveries were 93 ± 5.1% for acequinocyl and 78 ± 12% for acequinocyl-OH from 4 strawberry fruit control samples fortified separately with each analyte at 0.01-0.5 ppm (Table C.1). Apparent residues of acequinocyl and acequinocyl-OH were <0.01 ppm in/on all control strawberry fruit samples. The LOQ for both analytes is 0.01 ppm in/on strawberry fruit. The

LOD was not reported. Adequate sample calculations and chromatograms were provided.

In a total of 8 field trials conducted in the US during 2002, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications at a 21-day RTI to strawberries during the later stages of fruit development at ~0.30 lb ai/A/application, for a total of ~0.60 lb ai/A/season. Duplicate strawberry fruit samples were collected at 1 day after the last application in all seven trials and at 0, 1, 4, 7, and 14 days post-treatment in the residue decline trial.

Combined residues of acequinocyl and acequinocyl-OH were 0.145-0.350 ppm in/on 16 strawberry fruit samples harvested 1 day post-treatment (Table C.3). Average combined acequinocyl residues in/on strawberry samples declined from 0.427 ppm at 0 days post-treatment to 0.022 ppm at 14 days post-treatment.

Common cultural practices were used to maintain plants, and the weather conditions and the maintenance chemicals and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.1 Summary of Concurrent Recoveries of Acequinocyl from Strawberries.

Strawberry Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Fruit	0.01-0.5	4	88-100	93 ± 5.1	61-88	78 ± 12

TABLE C.2. Summary of Storage Conditions

Strawberry Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)[1]
Fruit	-32°C - (-2°C)	51-110	150

[1] The submitted stability data for acequinocyl and acequinocyl-OH residues in strawberry matrices stored frozen for up to 5 months (~150 days) are adequate.

TABLE C.3. Residue Data from Strawberry Crop Field Trials with Acequinocyl.

Trial ID (City, State/Year)	Region	Crop Variety	Total Rate, (lb a.i./A)	PHI (days)	Residues (ppm)[1]		
					Acequinocyl	Acequinocyl-OH	Combined[2]
Penn Yan, NY/	1	Northeastern	0.6	1	0.203, 0.197	<0.01, <0.01	0.208, 0.202
Johnston, SC/	2	Camarosa	0.6	1	0.236, 0.242	<0.01, <0.01	0.241, 0.247
Dover, FL/	3	Earlibrite	0.6	1	0.232, 0.140	0.0118, <0.01	0.244, 0.145
Kent City, MI/	5	Allstar	0.6	1	0.193, 0.195	<0.01, <0.01	0.198, 0.200
Porterville, CA/	10	Manteca Fern	0.6	14714	0.520, 0.318 0.341, 0.345 0.0233, 0.0392 0.0467, 0.0750 0.0144, 0.0192	0.0101, <0.01 <0.01, <0.01 <0.01, <0.01 <0.01, <0.01 <0.01, <0.01	0.530, 0.323 0.346, 0.350 0.028, 0.044 0.052, 0.080 0.019, 0.024
Exeter, CA/	10	Seascape	0.6	1	0.140, 0.179	<0.01, <0.01	0.145, 0.184
Hughson, CA/	10	Mt. Chandler	0.6	1	0.313, 0.227	<0.01, <0.01	0.318, 0.232
Forest Grove, OR/	12	Totem	0.6	1	0.202, 0.160	<0.01, <0.01	0.207, 0.165

[1] The LOQ for each analyte is 0.01 ppm.
[2] Residues <0.01 ppm (<LOQ) were estimated to be 0.005 ppm for calculation of combined residues.

TABLE C.4. Summary of Residue Data from Strawberry Crop Field Trials with Acequinocyl.

Commodity	Total Applic. Rate, (lb a.i./A)	PHI (days)	Residue Levels (ppm)					
			n	Min.	Max.	HAFT*	Mean	Std. Dev.
Acequinocyl Residues								
Fruit	0.3	12	16	0.14	0.345	0.343	0.222	0.063
Acequinocyl-OH Residues								
Fruit	0.3	1	16	<0.01[3]	0.0118	0.0084	<0.01	0.002
Combined Residues[4]								
Fruit	0.3	1	16	0.145	0.35	0.348	0.227	0.064

[1] HAFT = Highest Average Field Trial.
[2] The proposed PHI is 1 day.
[3] The LOQ for both analytes is 0.01 ppm in/on strawberries.
[4] For samples having analyte residues <LOQ, ½ the LOQ for the particular analyte was used for calculating the combined residues and average residues.

D. CONCLUSION

The strawberry field trial residue data are adequate and reflect a maximum application rate of 0.600 lb ai/A of acequinocyl (FlC) and a 1-day PHI.

E. REFERENCES

45651604.der1
45651606.der1

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.4.1/OPPTS 860.1520/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Processed Food and Feed - Apple

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 21-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45651604 Carringer, S. (2001) Magnitude of the Residue of Acequinocyl and its Metabolite in Apple Raw Agricultural and Processed Commodities. Lab Project Number: TCI-00-001. Unpublished study prepared by Morse Laboratories, Inc. 397 p.

EXECUTIVE SUMMARY:

In one trial, acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to apple trees at 1.21-1.22 lb ai/A/application, for a total of 2.43 lb ai/A/season. Applications were made during the later stages of fruit development. Apple fruit samples were collected at 14 days after the last application and subsamples were processed into wet pomace and juice using simulated commercial procedures. Samples were stored frozen for a maximum of 92 days, an interval supported by available stability data (45651604.der2).

Apple matrices were analyzed for residues of acequinocyl and acequinocyl-OH using a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse #Meth-133, Revision 3), which was validated and found to be adequate for data collection (45651604.der1). For this method, residues are extracted by homogenizing with acetonitrile (ACN) or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica-gel solid-phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC and quantified by MS/MS detection in the positive-ion mode. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on apple matrices. The limit of detection (LOD) was not reported.

Following two applications of acequenocyl (FlC) totaling 2.43 lb ai/A, combined residues of acequinocyl and acequinocyl-OH (expressed as parent equivalents) were 0.337 and 0.356 ppm in/on 2 apple fruit samples, 1.19 and 1.27 ppm in 2 wet pomace samples, and <0.01 ppm in 2 juice samples. The average processing factors of combined acequinocyl residues were 3.5x in wet pomace and 0.03x in juice. The maximum theoretical concentration factor for apples is >14x.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the apple processing study data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

TABLE A.1. Nomenclature of Test Compound and Metabolite

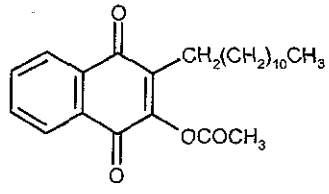
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties		
Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 acetone: >250 ethyl acetate: >250 xylene: >250 methanol: 6.1 n-heptane: 36 n-octanol: 29.2	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK$_a$)	no measurable pK$_a$	45434905
Octanol/water partition coefficient Log(K$_{OW}$)	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1. Study Use Pattern on Apple.

Location (City, State) Year	Application								Tank Mix Adjuvants
	Timing [1]	Formulation	Single Rate (lb a.i./A)	RTI [2] (days)	No. of Appl.	Method [3]	Volume (gal/A)	Total Rate (lb a.i./A)	
Sodus, NY, 2000	postemergence	1.25 lb/gal FlC	0.300 1.21-1.22	21	2	broadcast foliar	200	0.600 2.43	None

[1] All applications were made to trees during the later stages of fruit development (fruit 2-4").
[2] RTI = retreatment interval.
[3] All applications were made using ground equipment.

B.2. Processing Procedures

The apples used for processing were harvested from one trial location, 14 days following the second of two applications of acequinocyl (1.25 lb/gal FlC) totaling 2.43 lb ai/A. After collection, whole apple fruit samples were shipped unfrozen overnight to the processing facility (National Food Laboratory, Inc., Dublin, CA). Samples were stored at ca. 17 C at the processing facility for 1 day, and then processed using simulated commercial procedures into wet pomace and juice. After processing, subsamples of unwashed RAC, wet pomace and juice were immediately frozen (-15 to -2 C) and shipped within 2 days by overnight courier to the analytical facility (Morse Laboratories, Inc., Sacramento, CA).

B.3. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on apple fruit, wet pomace and juice was validated and found to be adequate for data collection (45651604.der1). A brief description of the method follows.

Residues are extracted by homogenizing with ACN or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix. Residues are then cleaned up by ACN:hexane partitioning and using silica gel SPE cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on all apple matrices. The LOD was not reported.

C. RESULTS AND DISCUSSION

The total frozen (-20 ± 5 C) storage intervals were 85-100 days for apple fruit, wet pomace and juice samples (Table C.2). The submitted stability data for acequinocyl and acequinocyl-OH residues in/on apple matrices stored frozen for up to 5 months (~150 days) was adequate (45651604.der2). The intervals from the apple processing study are supported by the available storage stability data.

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on apple matrices was validated and found to be adequate for data collection (45651604.der1). Method recoveries from apple fruit, juice, and wet pomace samples fortified with each analyte at 0.01 or 0.5 ppm averaged 84-96% for acequinocyl and 76-81% for acequinocyl-OH. For whole fruit, concurrent method recoveries were 96 ± 13% for acequinocyl and 78 ± 5% for acequinocyl-OH from six control samples of whole fruit fortified separately with each analyte at 0.01-2.0 ppm (Table C.1). For wet pomace, concurrent method recoveries were 83 ± 8% for acequinocyl and 71 ± 2% for acequinocyl-OH from three control samples of wet pomace fortified with each analyte at 0.01-2.0 ppm. For apple juice, concurrent method recoveries were 101% for acequinocyl and 86% for acequinocyl-OH from two control samples of juice fortified with each analyte at 0.01 and 0.5 ppm. Acequinocyl and acequinocyl-OH residues were <0.01 ppm in/on all control samples of the various apple matrices. The LOQ for both analytes is 0.01 ppm in/on apple fruit, wet pomace, and juice. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

Acequinocyl (1.25 lb/gal FlC) was applied as 2 broadcast foliar applications to apple trees during the later stages of fruit development at 1.21-1.22 lb ai/A/application, for a total of 2.43 lb ai/A/season. Apple fruit samples were collected at 14 days after the last application and subsamples were processed into wet pomace and juice using simulated commercial procedures.

Combined residues of acequinocyl and acequinocyl-OH (expressed as parent equivalents) were 0.337 and 0.356 ppm in/on 2 apple fruit samples, 1.19 and 1.27 ppm in 2 wet pomace samples, and <0.01 ppm in 2 juice samples (Table C.3). The average processing factors of combined acequinocyl residues were 3.5x in wet pomace and 0.03x in juice. The maximum theoretical concentration factor for apples is >14x.

TABLE C.1 Concurrent Recovery Results from Apple Fruit and Processed Apple Commodities for HPLC/MS/MS Method Meth-133.

Apple Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Whole Fruit	0.01-2.0	6	82-115	96 ± 13	70-83	78 ± 5
Juice	0.01, 0.5	2	92, 110	101	85, 87	86
Wet Pomace	0.01-2.0	3	76-91	83 ± 8	70-73	71 ± 2

TABLE C.2. Summary of Freezer Storage Conditions

Apple Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (days)[1]
Fruit	-20 ± 5	85	150
Juice		92	
Wet Pomace		86	

[1] The submitted stability data for acequinocyl and acequinocyl-OH residues in/on apple matrices stored frozen for up to 5 months (~150 days) were adequate (45651604.der2).

TABLE C.3. Residue Data from Apple Processing Study with Acequinocyl.

RAC	Processed Commodity	Total Rate (lbs ai/A)	PHI (days)	Residues (ppm)[1]			Processing Factor[3]
				Acequinocyl	Acequinocyl-OH	Combined[2]	
Apple	Unwashed Fruit (RAC)	0.600	14	0.056, 0.042	<0.01, <0.01	0.062, 0.048	NA
Apple	Unwashed Fruit (RAC)	2.43	14	0.331, 0.350	<0.01, <0.01	0.337, 0.356 (0.347)[3]	NA
	Wet Pomace			1.16, 1.24	0.029, 0.030	1.19, 1.27 (1.23)[3]	3.5x
	Juice			<0.01, <0.01	<0.01, <0.01	0.011, 0.011 (0.011)[3]	0.03x

[1] The LOQ for each analyte is 0.01 ppm. With the exception of the combined residues, the residues are expressed in terms of each analyte.

[2] The combined residues are expressed as acequinocyl equivalents (acequinocyl-OH residues multiplied by 1.12). Residues <0.01 ppm (<LOQ) were estimated to be 0.005 ppm for calculation of combined residues.

[3] The processing factor was calculated by the reviewer using the average combined residues (in parentheses) in the apple RAC and processed fractions.

NA = not applicable

D. CONCLUSION

The apple processing study data are adequate. In the trial conducted at an exaggerated rate, the average processing factors for the combined acequinocyl residues were 3.5x in wet pomace and 0.03x in juice.

E. REFERENCES

45651604.der1
45651604.der2

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329

Acequinocyl/PC Code: 006329/Arvesta
DACO 7.4.1/OPPTS 860.1520/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
Processed Food and Feed - Orange

Primary Evaluator	Sarah Levy, Chemist Registration Action Branch 1 (RAB1) Health Effects Division (HED; 7509C)
Approved by	George F. Kramer, Ph.D., Chemist RAB1/HED(7509C)

Note: This Data Evaluation Record (DER) was originally prepared under contract by Dynamac Corporation (20440 Century Boulevard, Suite 100; Germantown, MD 20874; submitted 24-FEB-2003). The DER has been reviewed by the HED and revised to reflect current OPP policies.

STUDY REPORT:

45651606 Carringer, S. (2002) Magnitude of the Residue of Acequinocyl and its Metabolite in Orange Raw Agricultural and Processed Commodities. Lab Project Number: TCI-01-003. Unpublished study prepared by Morse Laboratories, Inc. 329 p.

EXECUTIVE SUMMARY:

In one trial, acequinocyl (1.25 lb/gal FlC) was applied as two late season broadcast foliar applications to orange trees the later stages of fruit development at 1.18 lb ai/A/application, for a total of 2.36 lb ai/A/season. Orange fruits were sampled at 7 days after the last application and subsamples were processed into dried pulp, juice, and oil using simulated commercial procedures. Samples were stored frozen for a maximum of 56 days. The available storage stability data for residues in orange matrices are adequate (45651606.der1).

Orange matrices were analyzed for residues of acequinocyl and acequinocyl-OH using a high-performance liquid chromatography (HPLC)/mass spectrometry (MS)/MS method (Morse #Meth-133, Revision 3), which was validated and found to be adequate for data collection (45651604.der1) For this method, residues are extracted by homogenizing with acetonitrile (ACN) or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix, or with hexane (citrus oil and dehydrated pulp). Residues are then cleaned up using ACN:hexane partitioning, gel-permeation chromatography (GPC) (dehydrate pulp only), and silica gel solid phase extraction (SPE) cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC and quantified by MS/MS detection in the positive ion mode. The limit of quantitation (LOQ) for parent and acequinocyl-OH is 0.01 ppm in/on all matrices except citrus oil, which has a LOQ of 0.5 ppm for each analyte. The limit of detection (LOD) for all analytes in/on all matrices was not reported.

Following two applications of acequinocyl (FlC) totaling 2.36 lb ai/A, combined residues of acequinocyl and acequinocyl-OH (expressed as parent equivalents) were 0.293 and 0.241 ppm in/on 2 orange fruit samples, 0.318 and 0.263 ppm in 2 dried pulp samples, <0.01 ppm in 2 juice samples, and 44.5 and 43.7 ppm in 2 oil samples. The average processing factors for the

combined acequinocyl residues were 0.04x in juice, 1.09x in dried pulp, and 165x in oil. The maximum theoretical concentration factor for citrus is 1000x.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

The orange processing study data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document [DP Barcode D284757].

COMPLIANCE:

Signed and dated good laboratory practice (GLP), quality assurance, and data confidentiality statements were provided. No deviations from regulatory requirements were noted that would impact the study results or their interpretation.

A. BACKGROUND INFORMATION

Arvesta Corporation has submitted a petition proposing the use of acequinocyl (Kanemite™ 15 soluble concentrate (SC), 1.25 lb/gal FlC), a quinoline type of miticide, on citrus and pome fruits, strawberries, almonds and pistachios. There are currently no food/feed uses or tolerances for acequinocyl.

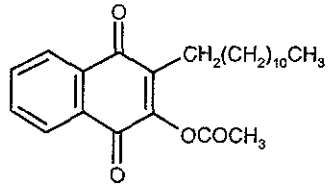
TABLE A.1. Nomenclature of Test Compound and Metabolite	
Compound	
Common name	Acequinocyl
Company experimental names	TM-413, AKD 2023
IUPAC name	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
CAS name	2-(acetyloxy)-3-dodecyl-1,4-naphthalenedione
CAS #	57960-19-7
End-use products/EP	1.25 lb/gal FlC

TABLE A.2. Physicochemical Properties		
Parameter	Value	Reference (MRID)
Melting point/range	59.6 C	45434906
pH	6.94	45434904
Density	1.13 g/cm^3	45434904
Water solubility (20°C)	6.69 µg/L	45434906
Solvent solubility (g/l at 20°C)	1,2-dichloroethane: >250 ethyl acetate: >250 methanol: 6.1 n-octanol: 29.2 acetone: >250 xylene: >250 n-heptane: 36	45434904
Vapor pressure at 25°C	1.69 x 10^{-9} pascals	45434905
Dissociation constant (pK$_a$)	no measurable pK$_a$	45434905
Octanol/water partition coefficient Log(K$_{OW}$)	≥6.2	45434906
UV/visible absorption spectrum (λmax, nm)	neutral pH: 242, 248, 262, 270, 335 acid pH: 242, 248, 265, 270, 330 basic pH: 232, 245, 255, 275, 362	45434905

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1. Study Use Pattern on Orange.

Location (City, State, Year)	Application								Tank Mix Adjuvants
	Timing [1]	Formulation	Single Rate (lb a.i./A)	RTI [2] (days)	No. of Appl.	Method [3]	Volume (gal/A)	Total Rate (lb a.i./A)	
Porterville, CA, 2001	postemergence	1.25 lb/gal FlC	0.30	21	2	broadcast foliar	53	0.600	None
			1.18					2.36	

1 All applications were made to trees at the immature fruit stage (2-4").
2 RTI = Retreatment Interval
3 All applications were made using ground equipment.

B.2. Processing Procedures

The oranges used for processing were harvested from one trial location, 7 days following the second of two applications of acequinocyl (1.25 lb/gal FlC) totaling 2.36 lb ai/A. After collection, orange fruit samples were shipped unfrozen overnight to the processing facility (The National Food Laboratory, Inc., Dublin, CA). Samples were stored at ca. 17 C at the processing facility for 1 day, and then processed using simulated commercial procedures into dried pulp, juice, and oil. After processing, subsamples of unwashed raw agricultural commodity (RAC), dried pulp, juice, and oil were immediately frozen (-20 to -8 C) and shipped within 6 days by overnight courier to the analytical facility (Morse Laboratories, Inc., Sacramento, CA).

B.3. Analytical Methodology

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on orange fruit, dried pulp, juice, and oil was validated and found to be adequate for data collection (45651604.der). A brief description of the method follows.

Residues are extracted by homogenizing with ACN or ACN:water (10:1 or 5:4, v/v), depending on the moisture content of the matrix, or with hexane (citrus oil and dehydrated pulp). Residues are then cleaned up using ACN:hexane partitioning, gel permeation chromatography (dehydrate pulp only), and silica gel SPE cartridges. Residues of parent and acequinocyl-OH are analyzed by reversed-phase HPLC using a mobile phase gradient of water to methanol (each containing 0.1% formic acid). Residues are detected and quantified by MS/MS detection in the positive ion mode. The transition of m/z 385 to 189 was monitored for parent and the transition of m/z 343 to 189 was monitored for acequinocyl-OH. The LOQ for parent and acequinocyl-OH is 0.01 ppm in/on all matrices except citrus oil, which has a LOQ of 0.5 ppm for each analyte. The LOD for all analytes in/on all matrices was not reported.

C. RESULTS AND DISCUSSION

The total frozen (-20 ± 5 C) storage intervals were 10-56 days for orange fruit, dried pulp, juice, and oil samples (Table C.2). The submitted stability data for acequinocyl and acequinocyl-OH residues in/on orange matrices stored frozen for up to 5 months (~150 days) were adequate (45651606.der1).

The HPLC/MS/MS method (Morse #Meth-133, Revision 3) for determining residues of acequinocyl and acequinocyl-OH in/on orange matrices was validated and found to be adequate for data collection (45651604.der1). Method recoveries from orange fruit, juice, dry pulp, and citrus oil samples fortified with each analyte at 0.01- 25 ppm averaged 80-101% for acequinocyl and 70-116% for acequinocyl-OH. With the processing study, average concurrent method recoveries were 84-103% for acequinocyl and 78-98% for acequinocyl-OH from 7 orange fruit, 1 juice, 1 dried pulp, and 2 oil control samples fortified separately with each analyte at 0.01-100 ppm (Table C.1). Acequinocyl and acequinocyl-OH residues were <0.01 ppm in/on all control orange fruit samples. The LOQ for both analytes is 0.01 ppm in/on orange fruit matrices, except oil, which has a validated LOQ of 0.5 ppm for each analyte. The LOD was not reported. Adequate sample calculations and chromatograms were provided.

Acequinocyl (1.25 lb/gal FlC) was applied as two broadcast foliar applications to orange trees during the later stages of fruit development at 1.18 lb ai/A/application, for a total of 2.36 lb ai/A/season. Orange fruit samples were collected at 7 days after the last application and subsamples were processed into dried pulp, juice, and oil using simulated commercial procedures.

Combined residues of acequinocyl and acequinocyl-OH (expressed as parent equivalents) were 0.293 and 0.241 ppm in/on 2 orange fruit samples, 0.318 and 0.263 ppm in 2 dried pulp samples, <0.01 ppm in 2 juice samples, and 44.5 and 43.7 ppm in 2 oil samples (Table C.3). The average processing factors of combined acequinocyl residues were 0.04x in juice, 1.09x in dried pulp, and 165x in oil. The maximum theoretical concentration factor for citrus is 1000x.

TABLE C.1. Concurrent Recovery Results from Orange Field Trials Trial for HPLC/MS/MS Method Meth-133.

Orange Matrix	Spiking Level (mg/kg)	Sample size	Acequinocyl		Acequinocyl-OH	
			Recoveries (%)	Mean Recovery ± SD	Recoveries (%)	Mean Recovery ± SD
Whole Fruit	0.01-1.0	7	72-100	86 ± 10	70-90	78 ± 8
Juice	0.01	1	84	NA	86	NA
Dried Pulp	0.5	1	103	NA	91	NA
Citrus Oil	0.5, 100	2	98, 104	101	87, 108	98

TABLE C.2. Summary of Freezer Storage Conditions

Orange Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Limit of Demonstrated Storage Stability (month)[1]
Fruit	-20 ± 5	10	approx. 5 months
Juice		27	approx. 3 months
Dried pulp		56	
Oil		22	

[1] The submitted stability data showed that acequinocyl and acequinocyl-OH residues in/on orange matrices were stored frozen for up to 5 months (~150 days) (45651606.der1).

TABLE C.3. Residue Data from Orange Processing Study with Acequinocyl.

RAC	Processed Commodity	Total Rate (lbs ai/A)	PHI (days)	Residues (ppm)[1]			Processing Factor[3]
				Acequinocyl	Acequinocyl-OH	Combined[2]	
Orange	Unwashed Fruit (RAC)	0.6	7	0.084, 0.048	<0.01, <0.01	0.090, 0.054	NA
Orange	Unwashed Fruit (RAC)	2.36	7	0.270, 0.224	0.020, 0.015	0.293, 0.241 (0.267)[3]	NA
	Juice			<0.01, <0.01[2]	<0.01, <0.01	0.01, 0.01 (0.01)	0.04x
	Dried Pulp			0.138, 0.114	0.161, 0.133	0.318, 0.263 (0.291)	1.09x
	Oil			37.9, 37.2	5.88, 5.82	44.5, 43.7 (44.1)	165x

[1] The LOQ for each analyte is 0.5 ppm in oil and 0.01 ppm in all other orange matrices. With the exception of the combined residues, the residues are expressed in terms of each analyte.

[2] The combined residues are expressed as acequinocyl equivalents (acequinocyl-OH residues multiplied by 1.12). Residues <0.01 ppm (<LOQ) were estimated to be 0.005 ppm for calculation of combined residues.

[3] The processing factor was calculated by the reviewer using the average combined residues (in parentheses) in the orange RAC and processed fractions.

NA = not applicable

D. CONCLUSION

In the trial conducted at an exaggerated rate, the average processing factors of combined acequinocyl residues were 0.04x in juice, 1.09x in dried pulp, and 165x in oil. The maximum theoretical concentration factor for citrus is 1000x.

E. REFERENCES

45651604.der1
45651606.der1

F. DOCUMENT TRACKING

RDI: S. Levy (21-APR-2004); G.F. Kramer (21-APR-2004); P.V. Shah (21-APR-2004)
Petition Number: 2F06440
DP Barcode: 284757
PC Code: 006329